Phenological, physiological, and ecological factors affecting the epiphyte *Notheia anomala* and its obligate host *Hormosira banksii*

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by

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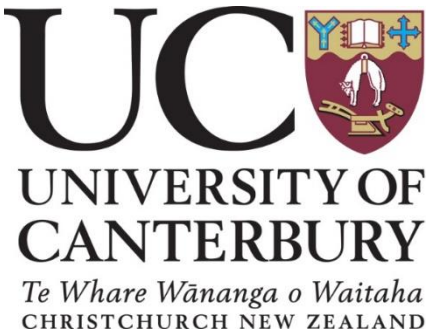

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Abstract

Notheia anomala is an obligate epiphyte commonly found on the abundant habitat-forming alga *Hormosira banksii* in intertidal areas throughout temperate Australasia. The tight co-evolved relationship between these species is unique because: (i) *Notheia* is a true obligate epiphyte, which is uncommon in the marine environment, (ii) the order Fucales is over 70 million years old and includes over 10 families, but *Notheia* is one of few fucoid epiphytes, and (iii) phylogenetically close species are rarely so closely linked (*Hormosira*, the obligate host of *Notheia*, is also a fucoid). This project is the first to address the phenological, physiological, and ecological factors affecting the *Notheia-Hormosira* relationship through a combination of field surveys and manipulative experiments.

Phenological observations indicated that the two species may have asynchronous life cycles. I found that *Notheia* reproduction peaked in April (Austral autumn) when seawater temperatures were mild, whereas previous studies have shown peak reproduction in *Hormosira* during the period July to October (Austral winter/spring). There were differences in the development of *Notheia* conceptacles across different habitats (high shore areas, low shore areas and tide pools). Conceptacles developed faster, and were at full maturity for longer in the tide pool habitat. It is likely that lower levels of desiccation stress in tide pools allow faster conceptacle development and longer periods of reproductive maturity.

From an evolutionary and ecological perspective, it is expected that the distribution of *Notheia* should closely resemble that of *Hormosira* across spatial and temporal scales. To test this, I compared distribution patterns of *Hormosira* and *Notheia* from the large continental scale to the small individual host plant scale. While *Notheia* biogeographical distribution is intricately linked to its host *Hormosira*, I found contrasting ecological habitat preferences, with tide pools hosting the lowest abundance of *Hormosira* and the highest abundance of *Notheia* respectively. At the host plant scale, I found that *Hormosira* plants from the high shore had the greatest number of *Notheia* clumps attached near the low-holdfast region. In the low shore and tide pools the pattern was opposite, with most *Notheia* clumps attached to the mid and high regions of the host. *Notheia* was equally likely to be found attached to male and female host plants, and more epiphytes were found attached to older than younger host plants.

Using field tagging and translocation experiments, I also quantified the survival and growth of *Notheia* at different densities exposed to various stressful environmental conditions. Tagged *Notheia* clumps, with different plant densities and sizes, from the low shore and tide pools all experienced high mortality over a five-month period associated with high dislodgement rates of the host

Hormosira. In translocation experiments of *Notheia* fronds (without its host), I found that individuals translocated to the high shore experienced close to 100% mortality, suggesting that desiccation and possibly photo inhibition are the main factors limiting the upward distribution of *Notheia*. Translocations to the low shore and tide pools demonstrated that *Notheia* fronds can survive and grow detached from its obligate host and suggest that the obligate dependency is most likely an early life stage requirement.

Finally, I tested whether the abundance of invertebrate inhabitants associated with *Hormosira* varies in the presence of *Notheia* across spatio-temporal scales. Field surveys showed that, as predicted, there were strong positive density-dependent effects of *Notheia* on both richness and abundance of invertebrates, regardless of the spatio-temporal context and resident invertebrate taxa, providing one of the first examples of a habitat cascade occurring in rocky intertidal systems.

Through a recolonization experiment, I tested whether invertebrate abundance was driven by (1) *Notheia* or *Hormosira*, (2) high or low amounts of *Notheia* and (3) living *Notheia* fronds or abiotic mimics. Hypotheses 1 and 2 were strongly supported, with more biomass of *Notheia* (as opposed to *Hormosira*) supporting more invertebrates, but not Hypothesis 3, as richness and abundances of inhabitants were similar between living *Notheia* fronds and artificial mimics. This suggests that *Notheia* is primarily providing habitat rather than food to the invertebrate inhabitants. Based on these results I hypothesized that invertebrates exert little or no grazing pressure on *Hormosira* and *Notheia*. This was tested in a laboratory food choice experiment focusing on potential grazing effects from herbivorous gastropods. In contrast to this hypothesis, I found negative effects of gastropods on both *Hormosira* and *Notheia*, with stronger grazing on *Notheia*. However, grazing rates were low overall and are likely to play only a minor role in regulating the abundance and distribution of the two species under natural field conditions. In support of the spatio-temporal surveys and colonization experiment, the grazing experiment also suggests that *Notheia* provide a better habitat for small invertebrates than *Hormosira*.

Seaweeds are key components of coastal ecosystems, providing habitat and food for a wide range of marine organisms. Therefore, understanding their life history patterns and reproduction dynamics is essential for managing coastal areas and assessing ecosystem health. This study is the first to explore the long-term phenology and periodicity of reproduction in *Notheia*. Furthermore, my results support a growing number of habitat cascade studies from different ecosystems, and suggest that these processes are common in marine benthic systems.

Chapter One

General Introduction

1.1. Introduction

The term ‘ecology’ was first described in the mid-1900s by the German biologist Ernst Haeckel as “the study of relationships between organisms and their physical environment”. The definition now encapsulates the relationships organisms have with their environment, as well as the relationships organisms have with one another, as many organisms exist in direct contact with one another over their life cycles. Interactions between organisms can be highly complex and often differ between species and environmental variables; therefore, describing species relationships can be difficult.

One complex type of interaction commonly found in nature is epibiosis, where one organism grows on another, typically without being parasitic (Wahl 1989). In epibiosis, ecological relationships between the ‘basiphyte’ (=host) and ‘epibiont’ (=species growing on the host) can be highly variable, and the nature of the relationship can change across ecological transition zones such as diurnal cycles, seasonal cycles, lunar cycles etc. (Thornber et al. 2016). The focus of my research is a common type of epibiosis called epiphytism, whereby a ‘plant’ lives on the surface of another plant, often harmlessly. In this introduction, I will first explain epiphytism from terrestrial systems and the basic terminology required to the understand epibiosis. I will then discuss marine examples of epiphytism, before I introduce my study species, the epiphyte *Notheia anomala* and its host *Hormosira banksii*.

1.1.1. Epiphytism

Epiphytes are plants that grow on other plants, deriving only physical support from their host (the basiphyte) while obtaining moisture and nutrients independently (Potin 2012). The term is derived from the Greek *epi-* meaning ‘upon’, and *phyton-* meaning ‘plant’. This growth pattern has evolved in numerous types of terrestrial plants, including mosses, lianas, vines, orchids, and bromeliads, and can be found in nearly all major groups in the plant kingdom from diverse habitats over the globe. Some epiphytic organisms can penetrate the cell walls of their hosts to extract resources, and these organisms are usually referred to as hemiparasites as they can obtain some resources from their host but also carry out photosynthesis (Potin 2012). Mistletoes are a well-known example of hemiparasites. They use a 'haustorium' to attach and penetrate the branches of their host to absorb water and nutrients, but are still capable of independent photosynthesis. Leonardi et al. (2006) listed five types of anatomical relationships that an epiphyte and its host can share:

> Infection Type I: Epiphyte is weakly attached to the surface of the host and is not associated with any damage of the hosts tissues,

Infection Type II: Epiphyte is strongly attached to the surface of the host and is not associated with any damage to host tissues,

Infection Type III: Epiphyte penetrates the outer layers of the host cell wall without damaging its cortical cells,

Infection Type IV: Epiphyte penetrates deep into the host cell wall and disorganizes its cortical tissues,

Infection Type V: Epiphyte penetrates deeply into the cortex of the host, reaches the medullary tissue, and causes destruction of host cells in the area around the attachment.

Epiphytic plants can be fundamental to ecosystem functioning as they often provide resources to a range of non-host species (Figure 1.1), such as providing nesting habitat for birds, and protection from predators for small mammals (Watson 2002). Epiphytes also add to the photosynthetic output of a system, provide digestible food to herbivores (as they often grow without lignin), serve as important food sources with nectar and fruits, and provide refuge for many prey invertebrate species. Ecologists have called these types of species interactions as ‘facilitation cascades’, and in particular ‘habitat cascades’. While the epiphyte serves as a fundamental structure in the system, the host is sometimes referred to as a ‘primary habitat former’ because it provides a basic habitat for epiphytes or ‘secondary habitat former’ to attach onto (Thomsen et al. 2010). Without the presence of the host, the secondary habitat former (if obligate to the host) would not be able to provide additional structure, complexity, and niche space for clients. In the example below, the host or ‘primary habitat former’ has indirect positive effects on clients (Figure 1.1).

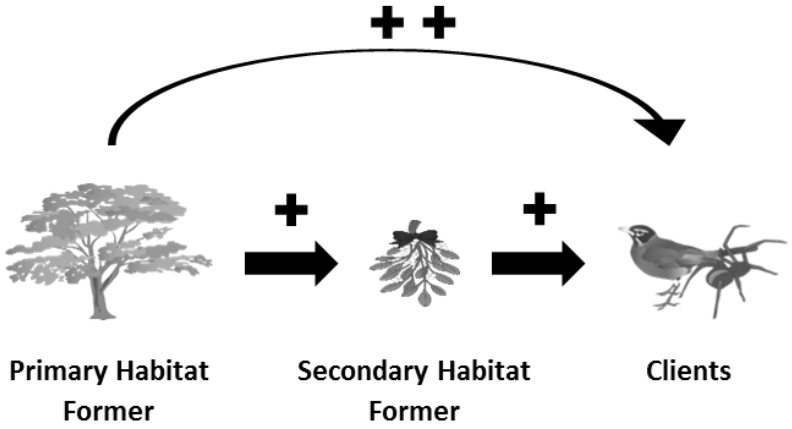

Figure 1.1. A schematic diagram illustrating how a facilitation cascade can occur. The primary habitat former is indirectly facilitating the 'clients' (organisms, typically mobile animals that depend on structural habitat-formers) through the direct facilitation of a secondary habitat former (in this case, a mistletoe) (Thomsen et al. 2010).

1.1.2. Marine epiphytes

Marine epiphytes (on substrates such as seagrasses and macroalgae) have been studied mostly in terms of species diversity and community functioning, with less emphasis on their distribution patterns or effects on their hosts. The relationship between epiphyte and host is variable and often determined by grazing pressure, desiccation tolerance, wave action, and the type of anatomical association (Potin 2012). Attachment strategies are different among species; for example, holo-epiphytes are attached to the outer layers of their hosts whereas amphi-epiphytes are deeply anchored into the tissues. The epiphyte can attach via just a single cell, form filamentous bases, or develop large rhizoidal structures (Potin 2012). Epiphytic assemblages on aquatic hosts can also vary by seasons or spatial distribution. For example the red alga *Polysiphonia lanosa* is an obligate epiphyte (meaning that it depends entirely on its host for survival) to the fucoid brown alga *Ascophyllum nodosum*, and its attachment is primarily to damaged tissues at wave-exposed sites, while mostly on the receptacles at sheltered sites (Levin and Mathieson 1991). This is why when studying epiphytic relationships, it is important to have a wide scope over temporal and spatial scales before drawing definitive conclusions.

A host may adapt to the presence of an epiphyte in at least three different ways: tolerance, avoidance, or defence (Wahl 1989). Therefore, an epibiotic association creates a complex network of benefits

and costs between the epiphyte and its host, which is often different and dependent on the species in question and the environment in which they are found (Figure 1.2).

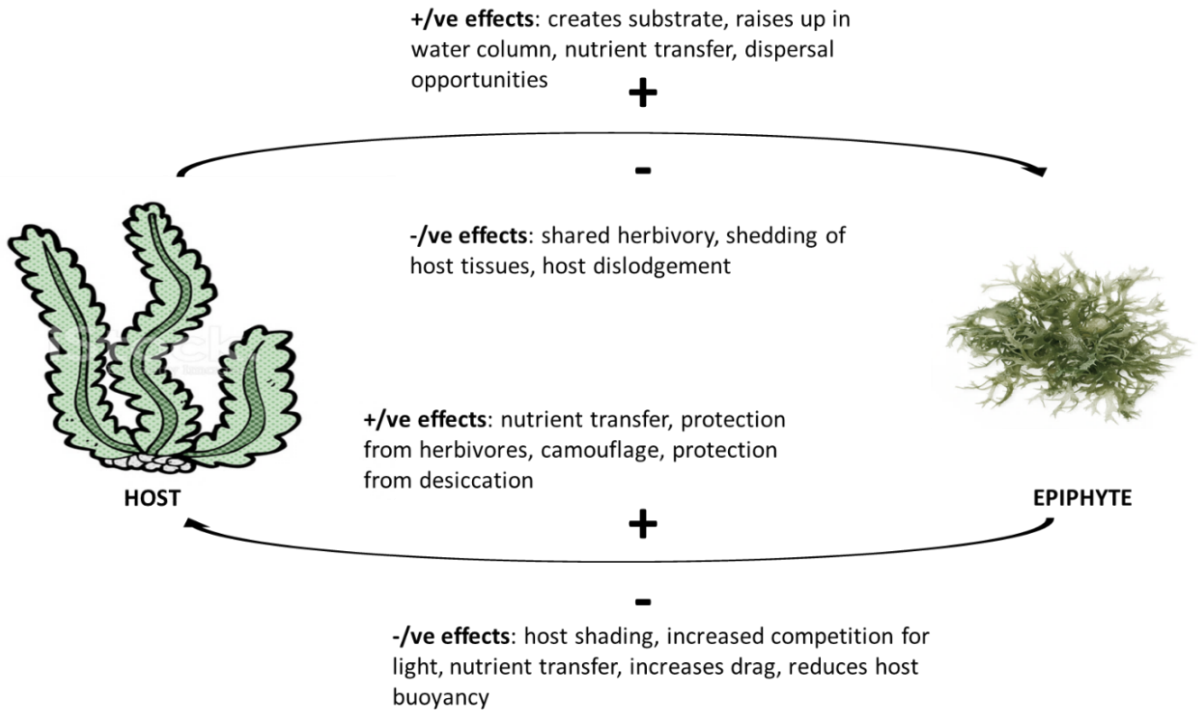

Figure 1.2. A schematic diagram summarising the costs and benefits of epiphytism for the host and epiphyte. For epiphytes, the benefits outweigh the costs, which is why epiphytism is such a dominant life form. For hosts, producing defences is costly, and many do not allocate a large amount of energy into epibiosis prevention.

1.1.3. Costs and benefits to hosts

A great number of species exhibit 'antifouling' properties where they keep their body surface largely clean of epibionts, suggesting there is a cost associated with epiphytism. Furthermore, old or damaged thalli often appear to be more susceptible to epiphyte colonisation because their inbuilt defences are decreased (Levin and Mathieson 1991, Potin 2012). However, it is unlikely that there are many sessile species that are not subject to epibiosis at some stage in their life cycle as any exposed, undefended substrate will ultimately become fouled in the marine environment (Wahl 1989). Producing defences is costly. For example, Larsson et al. (1986) observed that the relative concentration of defensive compounds in the small tree host *Salix dasyclados* was reduced when placed in nutrient-deficient conditions.

Competition for nutrients and light can increase greatly when an epiphyte attaches itself to a host; for example, shading levels of up to 80% have been reported (Sand-Jensen 1977, Wahl 1989). However, one study produced photosynthesis irradiance curves for the host *Odonthalia floccose* and concluded that the epiphyte *Soranthera ulvoidea* did not affect light acquisition in its host (Anderson et al. 2006). These contrasting findings suggest that costs can depend on the species and the environmental context. Furthermore, the attachment of dense calcareous epibiota such as bivalves or bryozoans can reduce the buoyancy of the host (Dixon et al. 1981). The algal epiphyte *Soranthera ulvoidea* increased hydrodynamic drag by around 50% causing the host seaweed *Odonthalia floccosa* to be more likely to break from the substratum (Anderson 2012, Anderson and Martone 2014). Finally, damage caused by herbivores that consume the epiphyte may also have adverse effects on the host (Dixon et al. 1981).

There may, however, also be benefits to the host from a symbiotic relationship with an epiphyte. Usually nutrient flow does not pass across the attachment area between an epiphyte and its host, but it has been suggested that energy flow between symbiotic partners may occasionally occur and ultimately benefit the host (Harlin 1973, Wahl 1989). The epiphyte may also provide protection from herbivores, or environmental stress such as desiccation. For example, when intertidal hosts with and without epiphytes were exposed to air during low tide, epiphytes doubled the time taken for hosts to lose 50% of their thalli moisture. Therefore, epiphytes may reduce physiological damage of their host by delaying desiccation (Anderson 2012). Epiphytes may also provide protection in the form of camouflage (Wahl 1989), and herbivores often prefer to graze on epiphytes rather than hosts - so the host could be benefitting by diverting herbivores away from host tissue and towards epiphytes (Anderson 2012).

1.1.4. Costs and benefits to epiphytes

Epiphytism is a successful life strategy, which is why we see its prevalence in many environments across the globe. Substrate availability is often a limiting growth factor for marine sessile organisms - and epiphytism probably evolved at least partly to counter this. Another benefit for the epiphyte is that it is raised higher in the water column by the host, and thereby increasing light levels for photosynthesis (Anderson 2012, Thornber et al. 2016). Other benefits include free transport and increased dispersal opportunities if the host is a mobile species (Wahl 1989) and, if the host is an animal, the epiphyte may be able to take up excreted nutrients from the host (Harlin 1973, Wahl 1989).

In nature it appears that with any form of benefit there must be a cost or trade-off. Epiphytism has obviously evolved as a highly successful life history strategy, but a variety of costs are associated with it. “Shared doom” between a host and its epiphytes can be seen for example, where mammalian seagrass consumers such as dugongs and manatees may consume the epiphyte while feeding on host seagrass shoots (Wahl and Hay 1995, Thornber et al. 2016). Costs also occur when the epiphyte increases drag on the host, potentially increasing dislodgement rates (Anderson 2012). Additionally, tissues of many perennial host species are shed during winter storm events, indicating that some hosts only provide substrata at certain times of year (Thornber et al. 2016).

1.1.5. Facultative epiphytism vs. obligate epiphytism

An epiphytic relationship can be described by the degree of reliance that the epiphyte has on its host. Most host-epiphyte interactions are facultative, whereby the epiphyte can attach to a range of host species. However, there are a few examples where an epiphyte attaches only to a few, or even a single host species. These are referred to as obligate epiphytes. For example, the red alga *Polysiphonia lanosa* is an obligate epiphyte that has only been found attached to the fucoid brown algal *Ascophyllum nodosum*. *P. lanosa* penetrates its host with rhizoids to obtain nutrition, demonstrating that this relationship is hemiparasitic, but the quantity of carbon obtained from its host is minimal (Harlin and Craigie 1975, Kim et al. 2002). A few other examples of obligate epiphytism in the marine ecosystem include the brown alga *Litosiphon laminariae* which grows exclusively on the brown alga *Alaria esculenta* (Rhys Williams 1965), and the red alga *Sonderella linearis* which is restricted to its host *Ballia callitricha* (Womersley 1965). While there are examples and publications, these close relationships between obligate epiphyte and host are overall very rare and poorly understood.

This thesis focusses on the relationship between the obligate epiphytic brown alga *Notheia anomala* and its host *Hormosira banksii*. This relationship is of interest because not only is obligate epiphytism uncommon in the marine environment, but both *Hormosira* and *Notheia* belong to the family of large brown algae Fucales, providing a rare example to study a host-epiphyte relationship between two closely related lineages (Gibson and Clayton 1987, Silberfeld et al. 2010).

1.2. Study species

Hormosira banksii is a fucoid brown alga that is abundant on rocky reefs in Australasia with low wave energy. The fronds of this species are made up of strings of spherical, gas-filled pneumatocysts (hereafter "nodes" or "beads"), which taper towards a small holdfast that is easily dislodged from the substratum (Schiel and Taylor 1999). However, this morphology enables the plant to tolerate heat stress and recover quickly from exposure during low tides (Lilley and Schiel 2006), enabling it to dominate the mid-high shore in many intertidal habitats. *Hormosira* produces eggs throughout the year but its peak reproduction is often confined to July-October (at least in northern New Zealand) when the sea temperature is around 14°C, as the viability of the eggs can be low in high sea temperatures such as 17-22°C (Begum and Taylor 1991, Dunmore 2006). The eggs are released directly into the surrounding water, and when fertilised they sink to the substratum to develop for several days before becoming attached (Schiel and Taylor 1999). *Hormosira* has been well-studied in New Zealand and Australia, and it is renowned for being an important habitat former that is fundamental to coastal ecosystem function.

Notheia anomala is an obligate epiphyte commonly found on *Hormosira* (although there is a single unconfirmed observation of *Notheia* being attached to *Xiphophora chondrophylla*) (Gibson and Clayton (1987), Raven et al. (1996); both papers use the same example). It appears *Notheia* growth entirely depends on being attached to *Hormosira*, as attempts to grow *Notheia* in culture were unsuccessful until *Hormosira* extracts were added (Hallam et al. 1980). Unlike for *Hormosira*, there are only a few studies on *Notheia* (Nizamuddin and Womersley 1960, Hallam et al. 1980, Gibson and Clayton 1987, Raven et al. 1995, Capon et al. 1998), and a Web of Science search of '*Notheia anomala'* comes up with only 48 results (January 2017), curiously most of which address biochemical analyses and phylogenetic tree-building. The ecological relationship between *Hormosira* and *Notheia*, and their effects on other species are therefore poorly understood. Also being poorly understood, is the species itself, with an early description of *Notheia* describing it as "a spurious thing", "anomalous in the extreme", and as an "abnormal growth of the nobler species *Hormosira*" (Harvey 1860).

Notheia provides a novel research opportunity. First, it is a true obligate epiphyte, which is uncommon in the marine environment. Second, although the Order Fucales is over 70 million years old it is one of the only fucoid epiphytes, and third, *Hormosira* is also a fucoid – which is rare to see in so closely related species (Silberfeld et al. 2010).

Research on this epiphytic species started in the 1800s when Harvey (1860) first (wrongly) described *Notheia* as a parasite, writing that it was inserted into the spore cavity within the beads of *Hormosira*. However, using the key written by Leonardi et al. (2006), it is now believed to be an Infection Type III epiphyte. Much later in the 1900s, Nizamuddin and Womersley (1960) showed that the reproductive micro- and macro-sporangia were produced in the walls of the mature conceptacles, and gave a detailed description of *Notheia* reproductive phenology. *Notheia* shares few traits with most species of Fucales (and even with most brown algae), which is why there was some early debate about which order it should be placed in. In particular, preliminary studies showed that its eggs are motile, which drove Nizamuddin and Womersley (1960) to make a thorough systematic investigation. They concluded that it should be classified as a distinctive type of the Heterogeneratae due to its different sporophytic and gametophytic generations. This classification did not stick however, and the species remains within the Fucales order despite its anomalies, forming its own distinctive Family *Notheia*ceae.

Notheia is monoecious, with male gametangia containing 64 spermatozoids and female gametangia containing 8 larger bi-flagellate motile cells. Female and male gametes fuse only after the female gametes have become attached to the surface of the host (Gibson and Clayton 1987). When Hallam et al. (1980) studied natural populations of *Hormosira* in Australia they found that tide pool populations had a consistently higher proportion of infected plants than the low-shore reef populations. This suggested that *Notheia* has a much narrower tolerance limit than its host. They found that sexually mature *Hormosira* plants carried more infections than juvenile plants, and the infections were most abundant on the reproductive conceptacles and usually close to the osteoles. When looking at the settlement preferences of *Notheia*, Hallam and colleagues discovered that it did not show any partiality towards a particular sex of its dioecious host. There have been no differing genotypic or phenotypic characteristics recorded between tide pool populations and low tide populations of *Notheia* (Raven et al. 1995), but *Notheia* has been recorded to have a higher photosynthetic capacity than its host *Hormosira*, which is probably due to its greater surface area or smaller allocation to chemical defences (Raven et al. 1995).

Figure 1.3. A photograph of the epiphyte *Notheia anomala* growing on its obligate host *Hormosira banksii*.

1.2.1. Costs and benefits

The costs to *Hormosira* of this epiphytic relationship are still unclear. Even though the tissues of *Hormosira* are pushed up against the thallus of *Notheia,* giving the impression that *Notheia* is emerging from deep within, there are no plasmodesmata observed between adjacent *Notheia* and *Hormosira* cells, but the association between the cells of the two species is very close and there is a wall-to-wall boundary (Hallam et al. 1980). Similarly, the benefits that *Notheia* obtains from attaching to *Hormosira* are also unclear. The fronds of *Hormosira* are weakly attached to the substratum and there is frequent dislodgement in storm events or periods of high wave energy. Therefore the fronds that drift may offer long-distance dispersal to *Notheia* which could be a key mechanism for the distributional success of this species (McKenzie and Bellgrove 2009). Capon et al. (1998) highlighted for the first time that tetrahydrofurans from *Notheia* act as potent and selective inhibitors of the larval development of parasitic nematodes, which may be a positive factor that *Hormosira* receives from this symbiotic relationship.

A phylogenetic study by Silberfeld et al. (2010) indicated that the species *Notheia anomala* appeared over 75 million years ago, whereas *Hormosira banksii* appeared around 30 million years ago. These findings suggest that *Notheia* may have once been a free-living species that has tightly co-evolved to utilise the benefits that epiphytism provides. The morphology of *Hormosira* allows it to dominate the mid-high intertidal zone of many sheltered coastlines, and it makes sense for a small species such as *Notheia* to be dependent on an organism that is largely successful and abundant. It is likely that *Notheia* propagules do not have to compete for settlement space due to *Hormosira* being present year-round. As a side note, this paper also demonstrated that *Hormosira* shares an immediate common ancestor with *Xiphophora chondrophylla* (the species in which *Notheia* can rarely be found attached to). Perhaps these species share similar chemical make-ups and other traits that are attractive to *Notheia* propagules.

This thesis should aid in understanding the ways in which this relationship is affecting *Hormosira* and *Notheia*, as well as their wider facilitative effects on invertebrate communities.

1.2.2. Why is the Hormosira-Notheia interaction of scientific interest?

Understanding the tightly co-evolved relationship between *Hormosira* and *Notheia* is of great scientific interest as it helps us to better understand host-specificity, host recognition, and host damage.

Not only will the information be filling a gap in our current knowledge of host-epiphyte relationships, but there are also practical ways to use the information. For example, Leonardi et al. (2006) used this type of data to reduce levels of epiphyte infection and diminish negative effects of epiphytes on their hosts on seaweed-farms. Furthermore, commercial harvesting of kelp species such as *Ecklonia maxima* can have negative effects on the habitat-forming epiphytes that are associated with it. Harvesters typically cut through the primary blades of the kelp which is where most epiphytes are found. Therefore a greater understanding of epiphyte ecology could encourage the development of new, non-lethal methods to harvest economically valuable species such as kelp so that they are not completely killed off (Anderson et al. 2006).

Analyses of *Notheia* biomass have shown a strong positive effect on invertebrate biodiversity (Thomsen et al. 2016). *Notheia* could be providing food, protection, and niche space for a suite of small invertebrates, an important group of organisms that again provide food for higher trophic levels. *Notheia* also has a higher photosynthetic capacity than its host *Hormosira*, probably because of its

greater surface area or smaller allocation to chemical defences (Raven et al. 1995). Therefore, when quantifying ecosystem health, it is important to take these factors into account, and consider how epiphytes are contributing to ecosystem productivity.

Epiphytes are ubiquitous in terrestrial and marine systems, and studying rare co-evolved obligate relationships such as the one between the algae *Hormosira* and *Notheia* will help researchers to understand the interesting life history strategy of epiphytism, so that we can better manage and preserve biodiversity.

1.3. Study aims

My thesis was divided into four broad questions addressing the phenological, physiological, and ecological properties of *Notheia*, and its interaction with its host and higher trophic levels:

1. When does *Notheia* reproduce?

This study will first quantify when *Notheia* reproduces. Qualitative information about reproduction in *Notheia* was first provided by Nizamuddin and Womersley (1960), and then by Gibson and Clayton (1987). However, these studies did not use well-replicated sampling and robust statistical analysis to document reproduction patterns. The findings from this core question are fundamental to the understanding of *Notheias* life history. I hypothesised that *Notheia* would be reproductive year-round, having male and female gametes present at all times. I also predicted that reproductive patterns would be different between high shore and low shore and tide pool populations due to different environmental pressures such as desiccation and photo-inhibition.

2. What factors influence *Notheias* distribution on its host?

It has been recorded that sexually mature *Hormosira* carry more infections than juveniles (Hallam et al. 1980), and data on the propagule settlement of *Notheia* did not show any preference towards a particular sex of its diecious host. I aimed to identify host-specific spatial patterns of *Notheia* individuals (i.e., quantifying attachment point and microscale attachment sites), to estimate effects of this epiphyte on its host (e.g., through increased drag or increasing shading). I predicted to find similar results to previous studies in regards to host-sex and host-age, and expected to find *Notheia* holdfasts only attached to the ostioles of *Hormosira* conceptacles.

3. What factors influence the distribution of *Notheia* in the intertidal zone?

It has been shown that *Notheia* has narrower tolerance limits than its host in terms of desiccation and therefore spatial distribution, as *Hormosira* individuals in constantly submerged tide pool populations have consistently greater proportions of infected plants than plants on exposed reefs (Hallam et al. 1980). I aimed to test mechanisms that control these large-scale distributions; for example, if desiccation is affecting *Notheia*s upper distribution, and if these stressors control growth processes. I also looked at whether *Notheia* growth is influenced by its placement in the canopy, host seaweed canopy cover, nutrient availability, and host seaweed species. Furthermore, I performed a long-term tagging experiment to document growth and longevity in *Notheia*.

I hypothesised that *Notheia* translocated to the high shore where desiccation risk is highest will not survive. I also hypothesised that once fully grown, it will survive if dislodged from *Hormosira*, and will still grow when manually tied to different host species due to its ability to independently photosynthesise. In the long term tagging experiment I expected that *Notheia* individuals would demonstrate 'weed-like' growth patterns, having high growth and turnover rates in at all tidal elevations.

4. How does the relationship between *Notheia* and *Hormosira* affect ecosystem functioning?

Thomsen et al. (2016) have demonstrated that *Notheia* has a strong positive effect on invertebrate diversity, and that these facilitative effects are stronger with more biomass. I tested this further by adding more replication, more regions, and more experimental work. I aimed to test what *Notheia* provides to the system in terms of resources, and tested the effect of grazing pressure on the epiphyte and its host.

In keeping with earlier work, I expected that *Notheia* will have positive effects on invertebrate biodiversity at all regions sampled, and the strength of these effects would increase with higher biomass. I also hypothesised that *Notheia* facilitates more invertebrates by providing food and niche space through its heavily branched morphology and small surface area compared to *Hormosira*.

1.4. Study sites

The main sampling regions were Kaikoura and Moeraki peninsulas, two of the largest algal-covered intertidal platforms in the central South Island of New Zealand (Schiel and Taylor 1999). Intertidal sites within these regions are characterised by flat rocky reefs with medium to low wave energy due to their protection by offshore reefs. Wairepo flat in Kaikoura is a gently sloping platform predominantly consisting of soft siltstone (Figure 1.4), and Moeraki Point is a platform made up of hard basaltic rock. The mid intertidal zone in these sites are dominated by the habitat former *Hormosira banksii*, which forms dense populations with often 100% cover (Schiel and Taylor 1999). Other regions used for this study include Cape Campbell in Marlborough, and Pile Bay on Banks Peninsula. These regions were selected to get widely separated sites with sheltered platforms where *Hormosira* is abundant (Figure 1.5).

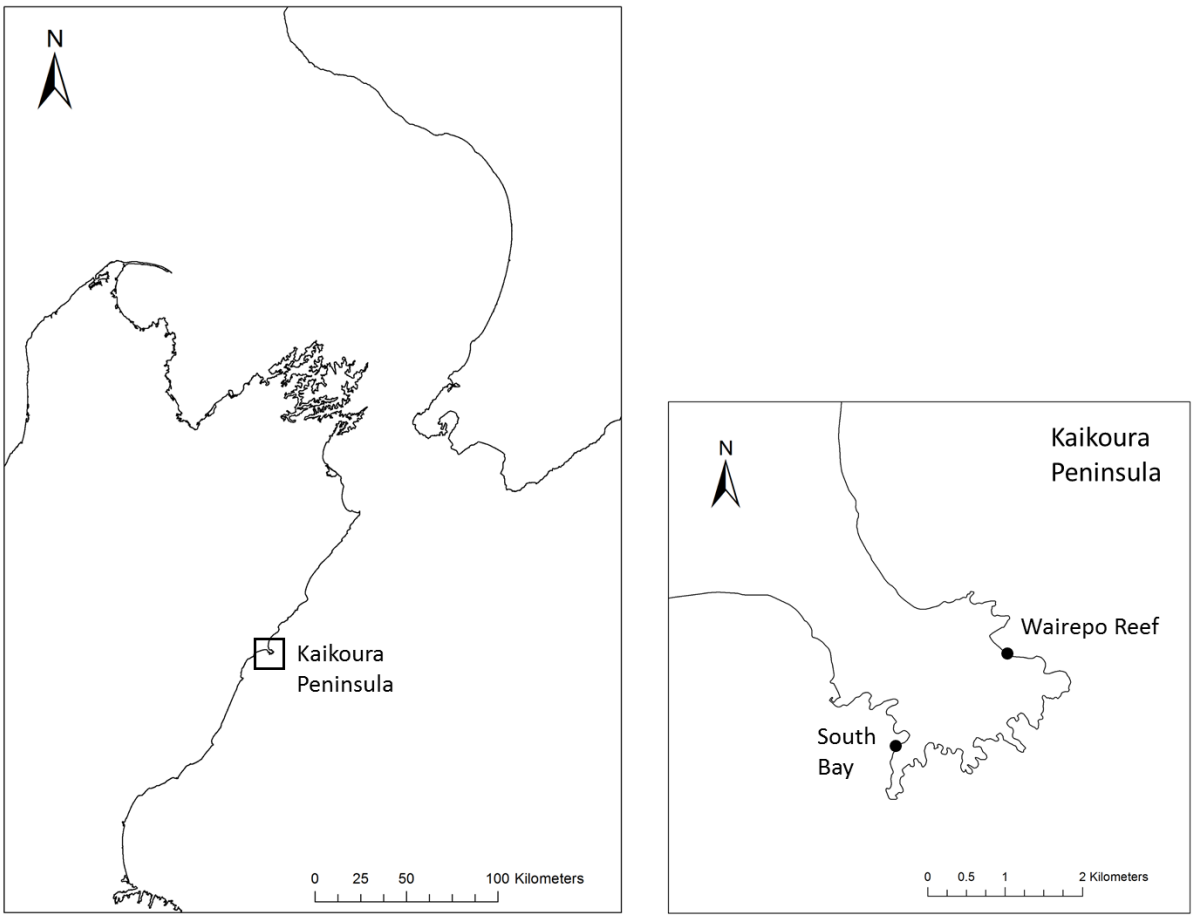

Figure 1.4. A section of the New Zealand map showing the distribution of Kaikoura on the North East coast of the South Island. The main sampling site for this project was at the Kaikoura Peninsula at Wairepo Reef, but some studies were also done at South Bay.

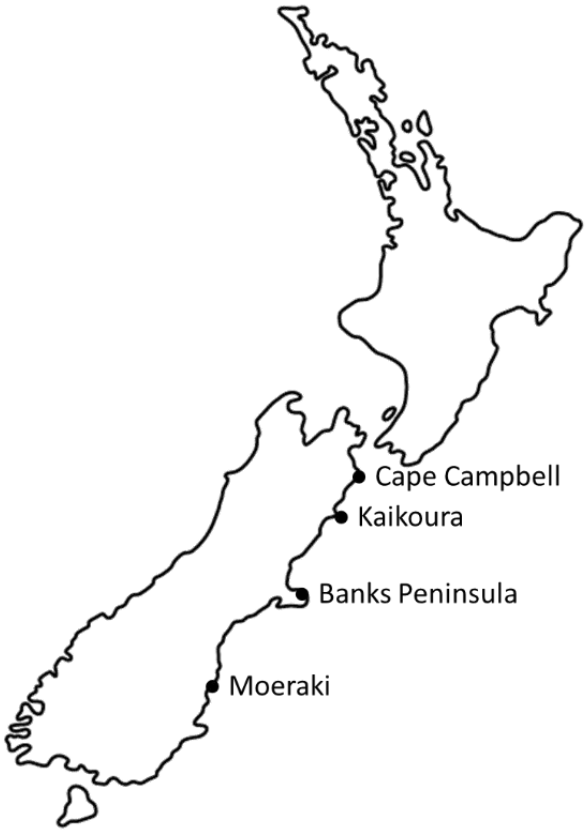

Figure 1.5. A map of New Zealand showing the four main sampling regions for this study. (1) Cape Campbell (-41.728981, 174.271525), (2) Kaikoura (-42.420553, 173.710327), (3) Banks Peninsula (-43.733446, 172.845579), and (4) Moeraki (-45.395288, 170.868468).

Chapter Two

The reproductive dynamics of *Notheia anomala*

2.1. Abstract

Seaweed is an important resource in rocky reef ecosystems, and understanding its life history patterns and reproductive dynamics is essential for managing coastal areas. Although many seaweed life history characteristics are generally similar, species that appear to be functionally and morphologically alike can demonstrate considerable differences in reproductive features. For example, the canopy-forming seaweed *Hormosira banksii* and its obligate epiphyte *Notheia anomala* are both brown algae within the Fucales, but they display large differences in reproductive strategies. *Hormosira* reproduction has been well-studied in the past, but little is known about the nature and periodicity of *Notheia* reproduction. This chapter aims to test whether *Notheia* is productive year-round, and whether individuals from different population across a tidal gradient show different timing of developmental stages. I sampled *Notheia* from low shore, high shore, and tide pool populations monthly for a year at a sheltered reef in Kaikoura. Using histological methods, these individuals were assigned to a reproductive stage based on the presence, abundance and development of male and female gametes within the reproductive conceptacles. From October 2015 to August 2016 *Notheia* reproduction peaked in April when seawater temperatures were mild. There were differences in development between the high shore, low shore and tide pools. It is likely that lower levels of desiccation stress in tide pools allows faster development and longer periods of reproductive maturity. Future studies should involve a quantitative method to distinguish differences between intertidal populations, and experiments assessing gamete release should be explored.

2.2. Introduction

Seaweeds are fundamental resources in coastal ecosystems, providing habitat and food for a wide range of marine organisms. Understanding their life history patterns and reproduction dynamics is therefore essential for managing coastal areas and assessing ecosystem health. Furthermore, knowledge about the periodicity and magnitude of reproductive events is crucial to understanding species distributional limits, recovery of populations, and ecological interactions (Brawley and Johnson 1992, Schiel 2006). One important resource that large seaweeds provide is habitat space that supports epibiotic species, and there are many seaweed species that cannot survive unless attached to a host's tissues (epiphytes). Typically, the life history traits of large host seaweeds (basiphytes) contrasts greatly to epiphytes, as they tend to

have slower growth, narrower temperature requirements, complex life histories, strategies that depend on sexual reproduction only, and perennial distribution patterns (Thornber et al. 2016). There are suggestions that basiphytes are much less resilient than epiphytes, and that they are harder to replace by a functionally similar species than epiphytes if local extinction was to occur. Epiphytes can be either host-specific (obligate) or non-host specific (facultative). Most in the marine system are facultative, being opportunistic and flexible about where their propagules settle and grow, and studies have shown that less than 5% of marine epibionts are obligate to a single plant or animal (Wahl 2008). Due to their rarity, it is likely that the reproductive strategies of obligate epiphytes will be different to facultative epiphytes. For example, obligate species may have their reproduction tightly synchronised with the host so that their propagules are guaranteed a substrate while host defences are low.

Fucoid life history characteristics are generally similar, although species that appear to be functionally and morphologically alike can demonstrate differences in reproductive features (Pearson et al. 1998, Berger et al. 2001, Steen and Rueness 2004). For example, species within the Order Fucales can show different variations in gametangia structure, with some oogonia being motile bearing flagella, and some being sessile (Brawley and Johnson 1992). Another difference in fucoids (and other Orders) is that species can produce gametes of both sexes in the same mature individual (dioecious), or species can only produce one type of gamete (monoecious). This is true for the dioecious habitat-former *Hormosira banksii* (hereafter *Hormosira*) and the monoecious obligate epiphyte *Notheia anomala* (hereafter *Notheia*), which are both within the Fucales, but which display quite different reproductive strategies. For example, differences in being monoecious and dioecious can have ramifications for fertilisation success and the genetic structure of their populations (Brawley and Johnson 1992). Reproductive periodicity can also differ greatly between functionally and morphologically similar species, as some species are reproductive year-round while others are seasonally constrained. *Hormosira* reproduction peaks in the colder months when sea surface temperatures are around 14°C (Begum and Taylor 1991), but little is known about when *Notheia* reproduces (Hallam et al. 1980, Gibson and Clayton 1987).

Notheia has recently been shown to have strong positive effects on the diversity of small invertebrates, and therefore also on secondary productivity and possibly positive effects on higher trophic levels (Thomsen et al. 2016). However, despite its potential ecological

importance and unusual life history, there is surprisingly little research available on the early life history traits of *Notheia*, such as reproduction and early settlement.

Notheia has male and female gametangia developing in the same conceptacle (Nizamuddin and Womersley 1960). Its macrosporangia and microsporangia are produced on the walls of developed conceptacles, and when released from the sporangia, both gametes are motile, with the females being much less active and settling rapidly. Gibson and Clayton (1987) were the first to culture *Notheia* successfully in the laboratory, so were able to add to the anatomical information given by Nizamuddin and Womersley (1960). The male and female gametangia have a three-layered wall, with microsporangia containing 64 zooids and macrosporangia containing 8 larger motile cells. Female gametangia are present throughout the year, but males have only been observed between April and July (Victoria, Australia). Many specific details of the gametangia, such as size, shape, and cell wall characteristics, are also described. They found that once released, male and female gametes freely intermingle, but males are not attracted to females while they are still motile. Female gametes will settle and reabsorb their flagella about 4h after release, which is when fertilisation will occur with a free-swimming male gamete. It is important to note that only *Notheia* females attached to *Hormosira* or other *Notheia* are able to form zygotes.

I aimed to test the timing and spatial variability of *Notheia* gamete development and release. I collected individuals monthly over a year and sampled at different tidal levels to test for environmental stressors. I hypothesised that *Notheia* would be reproductive year-round, having male and female gametes present at all times. I also anticipated that reproductive patterns would be different between high shore, low shore and tide pool populations due to different environmental pressures such as desiccation and photo-inhibition.

2.3. Methods

Small *Notheia* clippings, from the distal thallus region of the seaweed, were collected monthly from October 2015 to September 2016 at Wairepo Reef, Kaikoura. During each sampling period, fresh tissue was fixed within an hour of collection. Fixatives changed during the year; from October to April, the fixative was a sodium cacodylate-buffered glutaraldehyde solution, and from May to September a formalin/propionic acid solution (see appendix, Table A.1). Ten

individual clippings were randomly collected from three intertidal habitats; a 'high' zone (between 0.9 and 1.1 m above the lowest astronomical tide (LAT)), 'low' zone (between 0 and 0.5 m above LAT), and interspersed submerged rock pools (hereafter tide pools).

The specimens were placed in individual 7 mL vials and kept cool in chilli bins for transport back to the laboratory in Christchurch, where they were evacuated in a vacuum desiccator and left under vacuum pressure for at least 20 hours at room temperature in a fume cupboard. After the vacuum procedure, the specimens were manually rinsed in buffer, and dehydrated in an ethanol series to 70% ethanol (see appendix, Table A.2). A random subsample of five plants was selected for each shore habitat, and only those from every second month were further analysed to reduce processing effort. Individual samples were cut to fit into histology tissue cassettes, paying particular attention to suitable orientation of the branches for later longitudinal sectioning. The remaining alternate month samples were stored at 4°C for processing, if required.

The main samples were placed in an automated tissue processor (Shandon Citadel 1000, with ThermoFisher vacuum pump) and dehydrated in an increasing ethanol series, for approximately one hour at each concentration, until in absolute ethanol. They were automatically transferred into the "clearing agent", xylene, which occurred via a one-step 50% xylene/50% ethanol solution to absolute xylene (see appendix, Table A.3). Following three paraffin (Histosec) wax infiltrations (two under vacuum), the samples were finally set manually in fresh Histosec embedding wax using an embedding centre (Tissue-Tek, Miles Scientific). Once embedded, the blocks were sectioned longitudinally using a microtome (Leica RM 2165) and a Feather stainless steel microtome blade (S35). Two to four sections (10 µm thick) were transferred onto distilled water droplets on glass slides (pre-prepared with Haupt's adhesive) and bonded using a hot plate. Slides were dried in a drying oven at 37^{o} C for 1 day. The samples were stained manually using Haematoxylin and Eosin/Phloxine (see appendix, Table A.4, Table A.5). Immediately after the last xylene bath, a couple of drops of the mountant medium, Eukitt, were placed on the stained sections to avoid drying and then glass coverslips (22 x 50 mm) were applied. Slides were left to dry at room temperature in a fume cupboard.

Using a Nikon compound microscope, the slides were categorised into 6 different stages of development based on the presence or absence of male and female gametes. Multiple conceptacles were used per sample to estimate the stage. These categories were loosely based on the criteria used by Nutsford (2010) and Hill (2013). However, these studies were done on

animal tissue and were not inclusive enough for the stages I saw during the reproductive cycle. I therefore created my own criteria for staging reproduction in *Notheia*. These are shown in Figure 2.1. I was the only one who analysed the slides to avoid bias in assigning stages. Photomicrographs of representative features of each stage were taken with bright field illumination using a Zeiss AxioImager.M1 compound microscope, with a Zeiss AxioCam HRc CCD camera attached and AxioVision Rel. 4.8 software at 3900 x 3090-pixel resolution.

Due to the categorical nature of this data set, no statistics were performed. Categorical data such as 'reproductive stages' cannot be used as a response variable in tests examining the effects of factors (in this case month and shore elevation). Extensive graphing was used to draw conclusions on the pattern of *Notheia* reproduction.

Table 2.1. Criteria used to microscopically stage the development of *Notheia anomala* conceptacles. There were no stages which displayed only males.

Stage	Histological appearance
I.	Conceptacle is sterile. No evidence of male or female gametes. Often many paraphyses (sterile, hair-like filaments) can be seen.
II.	Only female gametes visible, but at very low numbers. The rest of the conceptacle is empty or contains empty sac-like structures. Paraphyses apparent.
III.	Only female gametes, with ~50% of the conceptacle being full.
IV.	Female and male gametes present, with ~50% of the conceptacle being full.
V.	Only female gametes visible, in large numbers. Conceptacle is often 80-95% full.
VI.	Female and male gametes can be seen in large numbers. Conceptacle is often 80-95% full.

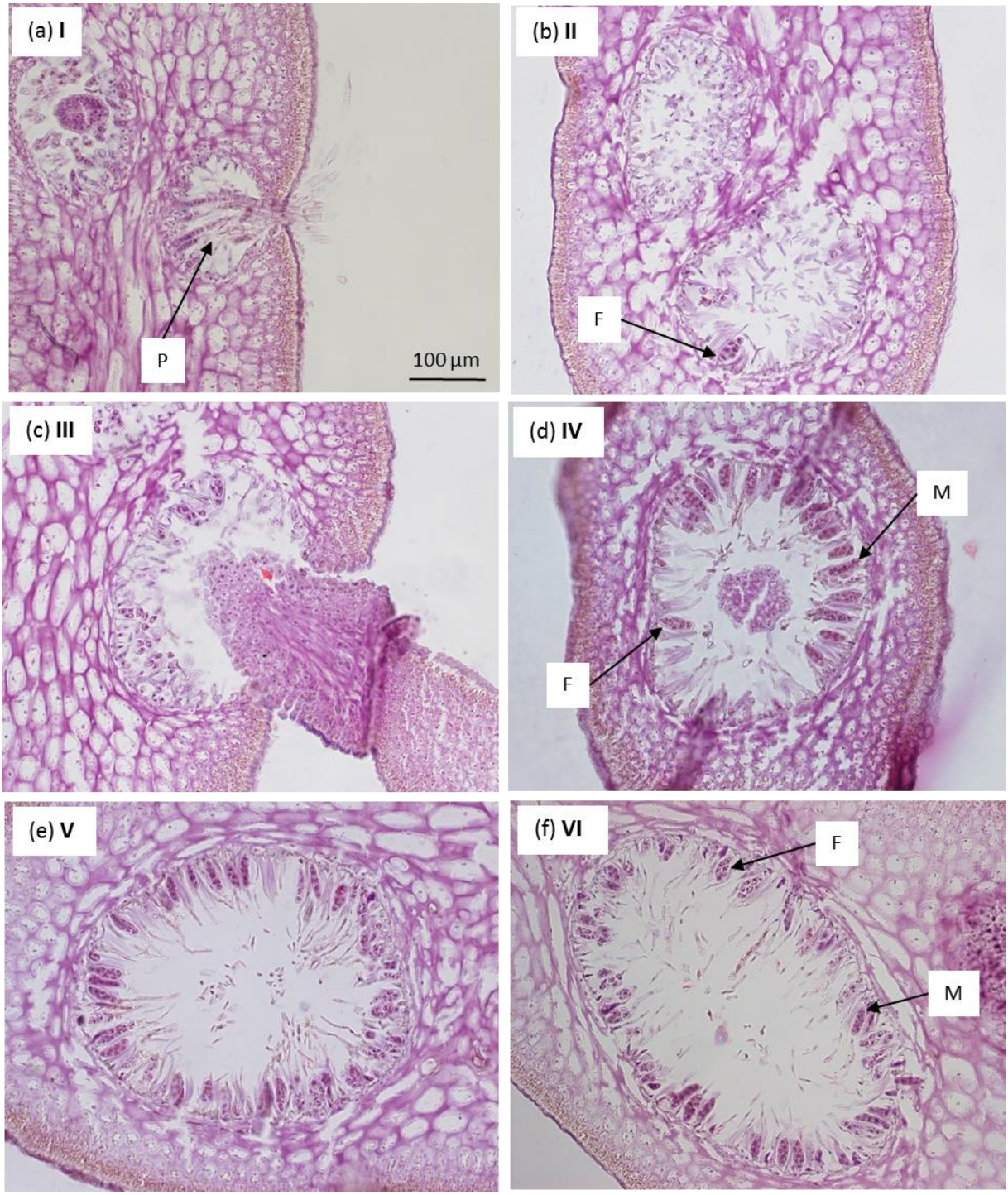

Figure 2.1. Light micrographs of *Notheia anomala* conceptacles at different stages of development. **(a)** Sterile - stage I, **(b)** few females – stage II, **(c)** 50% full of females – stage III, **(d)** 50% full of females and males mix – stage IV, **(e)** very full of females – stage V, and **(f)** very full of females and males mixed – stage VI. Also represented are paraphyses **(P)**, female gametes **(F)**, and male gametes **(M)**. Scale bar represents the magnification for all micrographs.

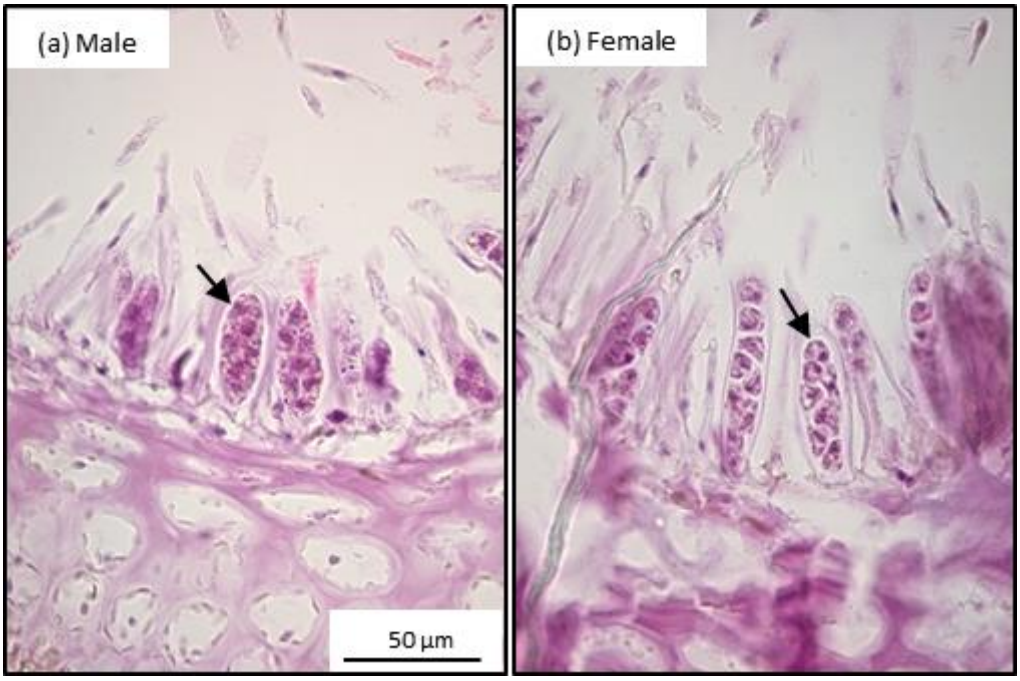

Figure 2.2. Higher magnification micrographs of **(a)** male, and **(b)** female gametangia (arrowed) from a *Notheia* individual living in a tide pool (April). Scale bar represents the magnification for both micrographs.

2.4. Results

Histological staging of *Notheia* conceptacles revealed distinct seasonal patterns in the presence and absence of male and female gametes during the sampling period of October 2015 to August 2016. Among the high shore, low shore, and tide pool habitats, there was a distinct quiescent period from July to October where most individuals had sterile conceptacles bearing only hair-like structures called paraphyses (Stage I) (Table 2.2, Figure 2.3, Figure 2.4). April demonstrated peak reproduction among habitats (Stage VI), as individuals sampled during this month had conceptacles filled >90% with both male and female gametes (Table 2.2, Figure 2.3, Figure 2.4). Stage II and III individuals occurred throughout the rest of the year, with stage IV and V generally appearing from December to June, but unlike Stage I and VI, there were large differences in the proportions of these intermediate stages of development among habitats (Figure 2.3, Figure 2.4).

During peak reproduction in April, the low shore populations had a lower proportion of individuals at stage VI (17% of individuals) than the high shore (60%) and tide pool (67%) populations (Figure 2.4). There was a peak in Stage V individuals in the high shore (40% of

individuals) and tide pools (100%) in December, but this peak was not observed in the low shore (Figure 2.4), Stage V was then present again in small proportions during April in the high (20%) and low shore (17%). Stage IV was highly variable across habitats, with two small peaks in the high shore in February and June, one large peak in the tide pools in February, and one small peak in the low shore in June (Figure 2.4). Stage III, where conceptacles were half filled with female gametes only, was never present in the tide pool population, with one peak in the high shore from November to January, and two peaks in the low shore in December and June (with December showing 100%). Stage II was also highly variable across habitats, but synchrony was ultimately observed in Stage I were all plants sampled appeared to be quiescent from July to October.

High shore individuals showed a higher proportion of conceptacles bearing male gametes for a longer period (December to July) (Figure 2.3a, Stage IV and VI) than the low shore and tide pool populations. The tide pools displayed males from January to June, and the low shore population had the shortest period bearing male gametes from March to July (Figure 2.3).

From October 2015 to August 2016 coastal seawater temperatures ranged between 12.5-16.5°C (Figure 2.5). The coolest temperatures were from March to April. There was an abnormal drop in temperature in November and December, which then gradually climbed back up to the warmest temperature which was in January (Figure 2.5).

Table 2.2. Number of *Notheia* individuals at each reproductive stage (Stage I to VI). Individuals from the high shore, low shore and tide pools are combined to show general patterns.

Stage	Oct	Dec	Feb	April	June	Aug
I	9	0	0	0	4	15
II	4	0	7	6	7	0
III	0	10	4	0	1	0
IV	0	0	3	1	2	0
V	0	6	0	2	0	0
VI	0	0	0	8	0	0
Total	13	16	14	17	14	15

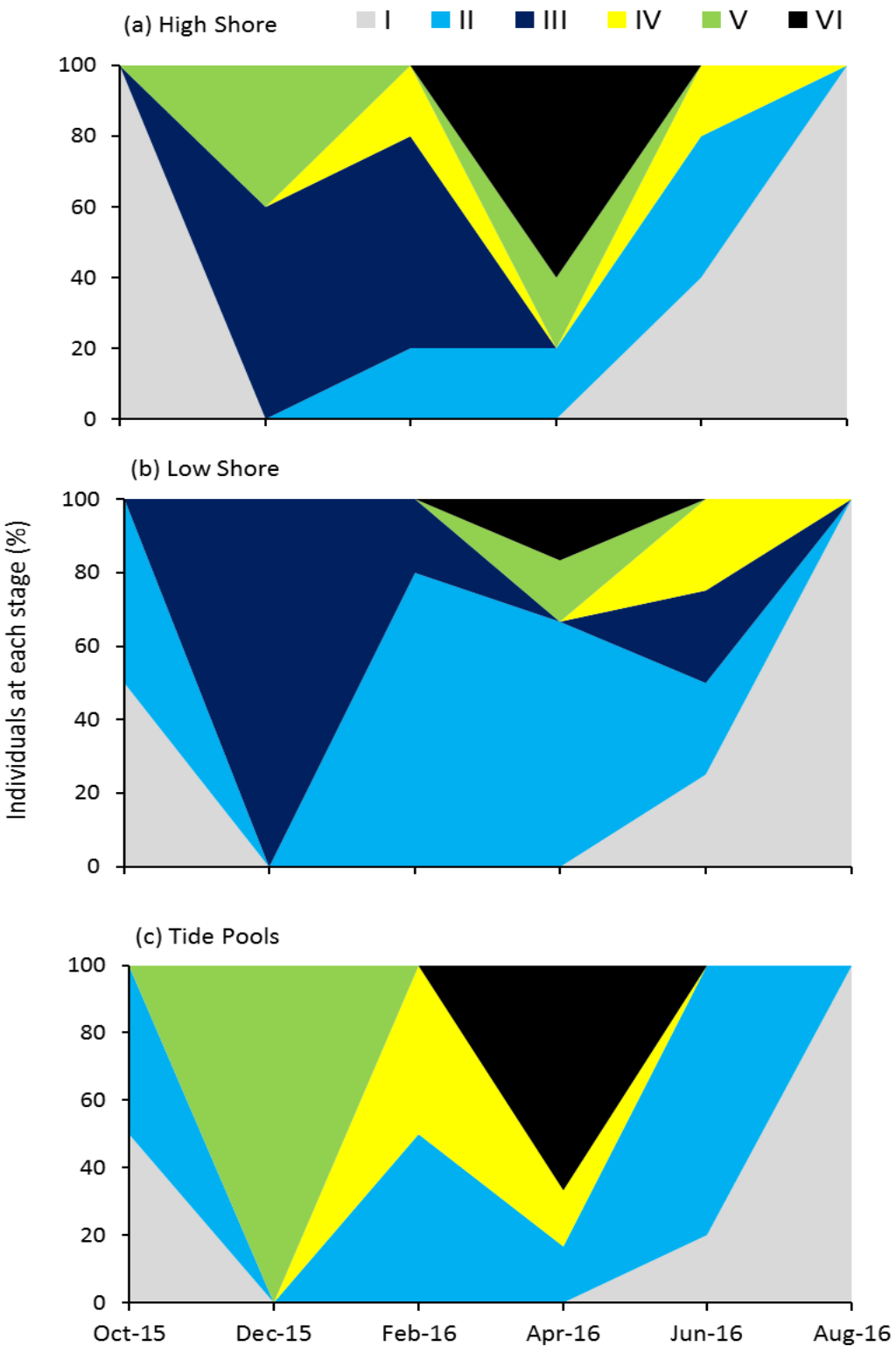

Figure 2.3. Proportion of *Notheia* individuals at each histological stage (as described in Table 2.1). (a) High shore (n=30), (b) low shore (n=31), and (c) tide pools (n=28). Values are shown as bimonthly from October 2015 to August 2016.

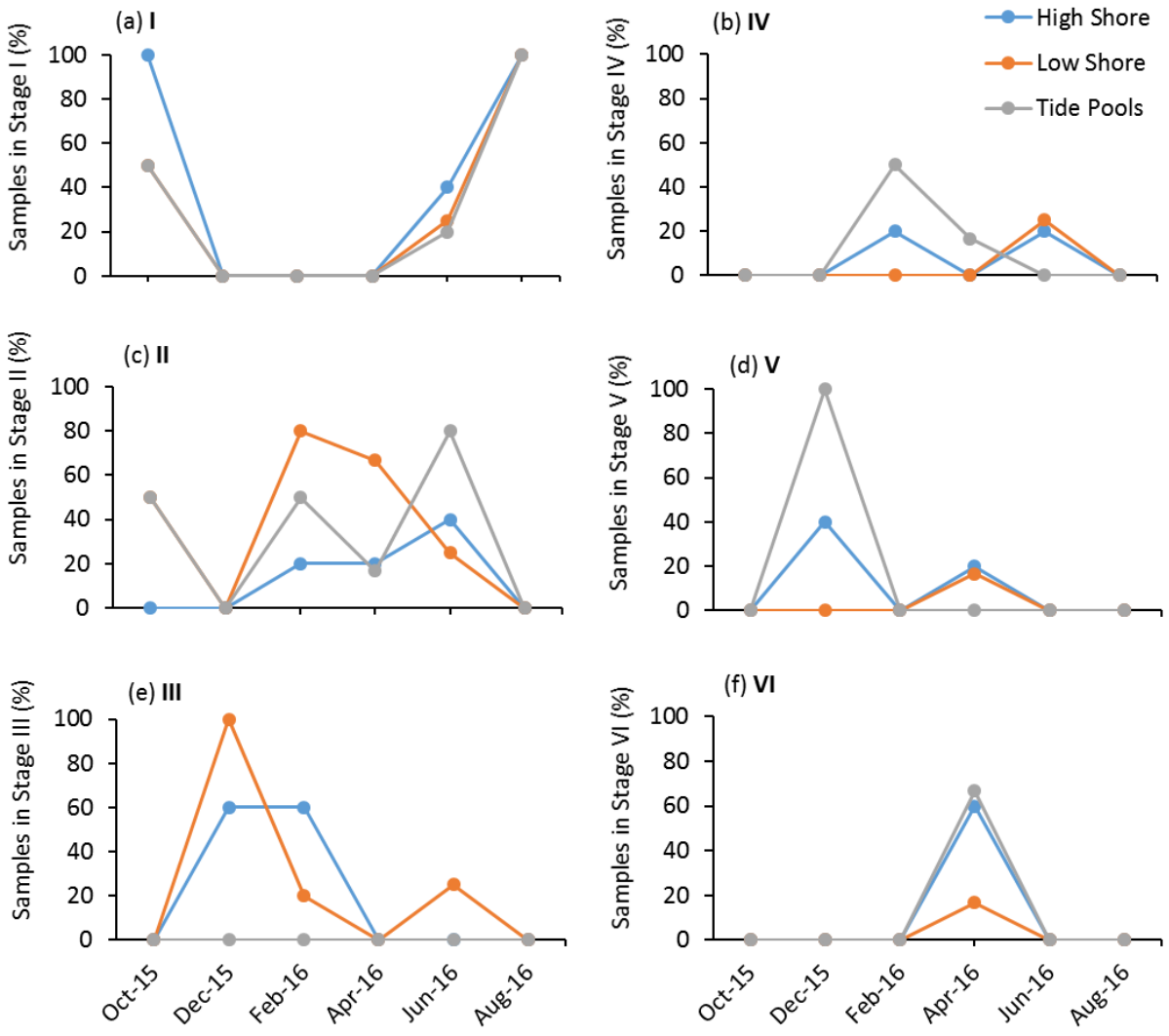

Figure 2.4. Reproductive stage differences between high shore, low shore, and tide pools over time. These graphs demonstrate key similarities and differences in quiescent and reproductive periods among populations.

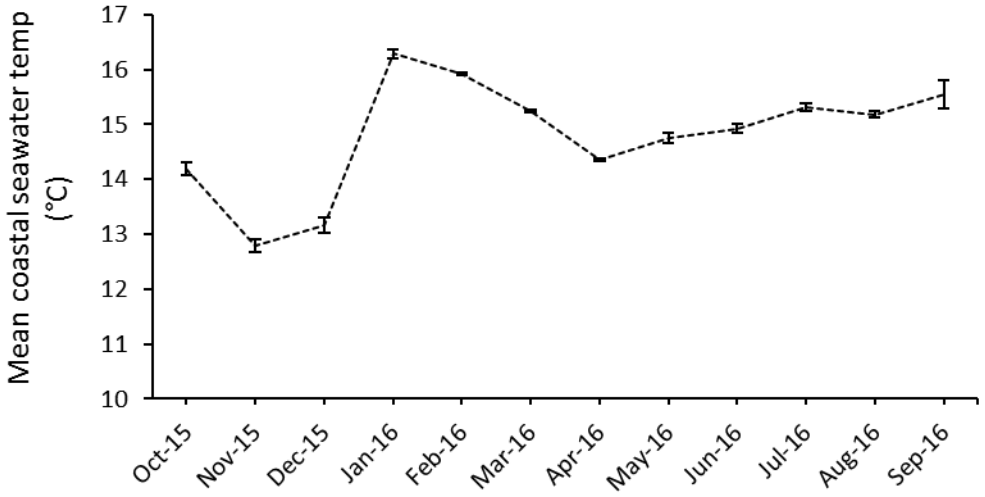

Figure 2.5. Average coastal seawater temperature (°C) at Kaikoura Peninsula from October 2015 to September 2016. Data collected from Onset HOBO temperature loggers. Error bars are standard error.

2.5. Summary

It was hypothesised that *Notheia* reproduction would occur year-round because it is a small weed-like species with high turnover rates and stable abundance across the year (Chapter Three). The seasonal patterns found in abundance and development of male and female gametes within the reproductive conceptacles was therefore surprising. As with most intertidal algae, these patterns were likely linked to coastal seawater temperature (Figure 2.5), as peak reproduction occurred in April when temperatures were mild (Stage VI), and a period of quiescence was observed from July to October when seawater temperatures are low (Stage I). Among the high shore, low shore, and tide pool habitats, this peak and drop in reproduction was matched over time, but the development (Stage II to V) in between was different in individuals growing in different habitats.

For example, there was a peak of Stage V individuals (conceptacles very full with only female gametes) in the high shore and tide pools in December, but this peak was not observed in the low shore. In Figure 2.5 it can be seen that there is a sudden drop in temperature in November and December, but then it rises again in January; it could be that these individuals in the high shore and tide pools were gearing up for the reproductive season too early due to those sudden abnormal temperature changes. Another example was in Stage III, which was never present in the tide pool population, with one peak in the high shore from November to January, and two

peaks in the low shore in December and June. These fluctuating differences between habitats may be due to varying levels of environmental stress (such as desiccation); for example, tide pool *Notheia* populations receive the smallest amount of desiccation stress and are therefore able to allocate more energy to reproduction for longer. Furthermore, the high shore habitat is the most stressful environment, which could explain why the individuals growing there had similar reproductive patterns to those in the tide pools. When plants are stressed, they will apportion more energy into reproduction to ensure survival of the species.

While these results help to describe the periodicity and nature of *Notheia* reproduction, overall sample sizes are relatively small, and therefore, future studies should include a sampling design with higher replication, more sites, and a thorough statistical procedure to test differences between habitats.

Chapter Three

The distribution and interactions of the obligate epiphyte *Notheia anomala* and its canopy forming host *Hormosira banksii*

3.1. Abstract

Epiphytes are plants attached to biogenic substrates. From an evolutionary and ecological perspective, it is expected that the distribution of epiphytes closely resembles their biogenic substrate across space and time. However, few studies have compared such co-distributional patterns across scales. Here, I compare distribution patterns of the common habitat-forming alga *Hormosira banksii* and its obligate epiphyte *Notheia anomala* from the continental scale to individual host plant of less than 30 cm. Field experiments were used to quantify the stability, survival and growth of *Notheia* at different levels of environmental stress. While *Notheia* biogeographical and host plant distribution is intricately linked to its host *Hormosira*, I found contrasting ecological habitat preferences, with *Hormosira* being least and *Notheia* most abundant in tide pools. These local scale differences are likely controlled by desiccation stress, as *Hormosira* is one of the most desiccation-resistant canopy formers in temperate Australasia, allowing it to dominate higher reaches of the intertidal zone than its epiphyte.

On the host plant scale, I found that *Hormosira* from the high shore had the greatest number of attached *Notheia* clumps near the low-holdfast region of the host, a pattern opposite to the low shore and tide pools where most *Notheia* clumps were attached on the mid and high regions of the host. These patterns potentially reflect small scale patterns within the host canopy in moisture levels, desiccation stress and available sunlight. Finally, *Notheia* was equally likely to be found attached to male and female host plants, and more epiphytes were found attached to older than younger host plants.

Tagged *Notheia* clumps, representing different densities and sizes, from the low shore and tide pools all experienced high mortality over a five-month period associated with high dislodgement rates of the host *Hormosira*. In translocation experiments of *Notheia* fronds (without its host) I found that when translocated to the high shore, almost all *Notheia* died (100% biomass loss), suggesting that desiccation (and perhaps photo inhibition) is the main factor that limits the upward distribution of *Notheia*. I also translocated *Notheia* to the low shore and artificially attached it to different hosts. This assay demonstrated growth of *Notheia* when placed in a protective mesh bag (where fragments are retained). This result demonstrated that *Notheia* fronds, indeed can survive and grow detached from its obligate host and suggests that the obligate dependency most likely is an early life stage requirement.

Comparing distribution patterns of *Notheia* from continental scales to the cm-level attachment point on the host, will aid ecologists and population biologists to better understand mechanisms that control

epiphytism. This information can then be used when assessing ecosystem stability and diversity over time, as epiphytes are key providers of food and refuge in many habitats.

3.2. Introduction

Epiphytes are plants that grow attached to other organisms, and they span many taxonomic divisions, such as mosses, lichens, ferns, cacti, orchids, liverworts and algae. They are an ecologically important component of many ecosystems (Edgar and Robertson 1992, Ellwood et al. 2002, Ellwood and Foster 2004), habitats (Hall and Bell 1988, Martin-Smith 1993, Thomsen et al. 2016), from tropical (Ødegaard 2000), to polar regions (Jennings and Steinberg 1997), and are sometimes recognised as ecosystem engineers, foundation species and secondary habitat-formers (Thomsen et al. 2010, Lobelle et al. 2013). Epiphytes are particularly common in shallow marine systems, perhaps because of severe light and space limitations in these environments. In such limited conditions, the benefits of epiphytism, such as access to light and space, and decreased grazing (associational defence), typically outweigh the costs, such as competition for nutrients, increased susceptibility to storms, and sometimes increased grazing through "shared doom", shaping the evolution and co-evolution of epiphyte-host relationships worldwide (Wahl 1989, Wahl and Hay 1995, Thornber et al. 2016).

Although most epiphytes have wide host specificities (Wahl and Mark 1999, Wagner et al. 2015, Thornber et al. 2016), a few have evolved narrow specificities, the most extreme example being 'obligate' epiphytism, where an epiphyte can only attach to a single host species. True obligate epiphytes are uncommon across ecosystems (Fernandez et al. 2010), but a few have been documented from the marine environment (Hughes et al. 1991, Levin and Mathieson 1991, Notoya and Miyashita 1999, Anderson et al. 2006). Furthermore, most obligate epiphytes are evolutionarily distant from their hosts (Hughes et al. 1991, Anderson et al. 2006), and close obligate co-evolution between two closely related species is rare.

One example of an obligate epiphytic relationship is the fucoid alga *Notheia anomala* that is only found attached to another fucoid, *Hormosira banksii* demonstrating that even archetypic non-epiphytic species can evolve epiphytic life-strategies (Silberfeld et al. 2010). *Hormosira* (the host organism) is a key habitat-forming species that is abundant on many wave-protected intertidal reefs and among mangroves in southeast Australia and New Zealand. *Hormosira* is a canopy forming species that can increase spatial complexity, alter local physical conditions, and facilitate a diverse range of mobile and sessile organisms (Lilley and Schiel 2006, Bishop et al. 2009). Many studies

have quantified biotic and abiotic factors affecting *Hormosira* populations (and vice versa) to understand its ecological function (Begum and Taylor 1991, Bellgrove et al. 1997, Underwood 1998, Lilley and Schiel 2006, Goodsell and Underwood 2008, McKenzie and Bellgrove 2009). By contrast, very little is known about the ecology and distribution of its obligate epiphyte *Notheia*.

I am only aware of two studies that have quantified distributional population dynamics of *Notheia*. Hallam et al. (1980) made monthly collections of fronds of *Hormosira* with attached *Notheia* from Sorrento (Victoria, Australia) over period of 6 months (from March to August) from tide pools and low shore populations. They found that epiphytism rates were much greater on *Hormosira* in tide pools than on the low shore, suggesting *Notheia* has low tolerance to desiccation stress. However, these results were only documented from a single site, over two seasons, and did not sample *Notheia* populations from the mid-high shore or the distribution of its essential substrate, *Hormosira*. Similarly, Thomsen et al. (2016) quantified distribution from a single site, but noted that the distribution patterns of *Notheia* and *Hormosira* were opposite, with *Hormosira* and *Notheia* being least and most abundant in tide pools, respectively. Furthermore, neither of these studies have quantified the ecological performance, such as survival and growth, of *Notheia* under field conditions.

Assessing the small-scale attachment point details of *Notheia* is also fundamental to understanding its ecology. For example, if there is a preference towards the sex or age of its host, and whether host specificity is only a prerequisite for the initial settlement and growth of *Notheia*. Furthermore, small (low density) *Notheia* clumps may have less of a hydrodynamic drag effect on its host than large (high density) clumps, and this may then have an effect on the size distribution of the population as a whole. Growth in *Notheia* may also be density-dependent, with smaller clumps typically gaining biomass and larger clumps losing biomass to hydrodynamic drag and grazers.

To address these research gaps, I, (i) quantified the distribution and abundance of *Notheia* and *Hormosira* at different spatio-temporal scales and, (ii) conducted field experiments to quantify the ecological performance of *Notheia* (survival and growth) under different environmental conditions.

For the spatio-temporal distribution, I hypothesized that;

(a) *Notheia* and *Hormosira* have contrasting elevational distributions (as in Thomsen et al. (2016),

(b) these distributions are consistent across regions,

(c) *Notheia* (a small epiphyte with high surface to volume ratios) has greater seasonal variation in biomass than *Hormosira* (a large canopy-forming perennial host with low surface to volume ratios),

(d) *Notheia* is more abundant on older (lower) than newer (higher) parts of the host (because it takes time for propagules to find and attach to a host) (see Figure 3.3 for examples),

(e) *Notheia* does not show preference towards the gender of its host, and

(f) *Notheia* holdfasts are only attached to the ostioles of *Hormosira* conceptacles as indicated in (Harvey 1860).

Furthermore, for the experiments, I hypothesised that;

(a) small 'clumps' of *Notheia* that are characterized by low hydrodynamic drag, survive longer than large clumps with high drag,

(b) *Notheia* growth is limited, for example by grazers and hydrodynamic drag, so that large clumps typically lose biomass whereas small clumps are more likely to gain biomass,

(c) the distribution of *Notheia* is limited by desiccation and it will therefore die if translocated into higher elevations on the shore, but will survive if translocated to the low shore and tide pools,

(d) that *Notheia* is host specific on *Hormosira* during its initial attachment and early life stages, but that adult fronds can grow, without hosts or associated with other canopy forming host species

(e) and growth of *Notheia*, like many other finely branched epiphytes with high surface to volume ratio is nutrient limited (Harrison and Hurd 2001, Hughes et al. 2004).

3.3. Methods

3.3.1. Global Distribution

In order to assess the distribution of *Hormosira* and *Notheia*, spatial coordinates were extracted (6th June 2016) for all herbarium collections of both species from the Australian Virtual Herbarium (https://avh.chah.org.au) and New Zealand Virtual Herbarium (www.virtualherbarium.org.nz). Coordinates were plotted on maps and analysed graphically for distribution limits and overlap in ranges.

3.3.2. Distribution and abundance on vertical, regional, and temporal scales

The distribution of *Hormosira* and *Notheia* was quantified from *Hormosira* beds at a 'high' zone (between 0.9 and 1.1 m above the lowest astronomical tide (LAT)), 'low' zone (between 0 and 0.5 m above LAT), and interspersed submerged rock pools (hereafter tide pools). For each elevation zone, percent cover of *Hormosira* and *Notheia* was quantified from 10 haphazardly placed 0.25 m^2 quadrats divided into 100 squares. This sampling procedure was first conducted in summer 2013 (December) at two regions, separated by 500 km of coastline; the Moeraki peninsula (-45.3567, 170.8602), and Kaikoura peninsula (-42.4200, 173.7103). I also sampled Kaikoura seasonally, with additional sampling in fall 2015 (March), winter 2015 (July), and spring 2015 (November).

Data were analysed with factorial analysis of variance (ANOVA) as 3 elevations x 2 regions, and 3 elevations x 4 seasons, with elevation and season as fixed factors, and region as a random factor. Most test factors had homogenous variances or low variance heterogeneity (Levene's test $p>0.05$, respectively see Table 3.2, Table 3.3). Analysis was done on untransformed data (as ANOVA is robust to minor heteroscedasticity for factorial designs with high sample sizes) thereby simplifying interpretations of significant interaction effects (Quinn and Keough 2002). Significant ANOVA results were followed by *post hoc* tests (Least Significant Difference (LSD)) to identify differences between treatments.

3.3.3. Distribution and abundance on host plant scale (point of attachment)

To test if *Notheia* attachments grow only on particular locations of the host plant, epiphytised *Hormosira* plants were removed from the base of its singular holdfast, and in the laboratory, *Notheia* clumps visible to the naked eye growing on the high, mid, and low region of the host were counted (Figure 3.1). *Hormosira* samples were collected in spring (2013), summer (2013, 2014), and fall (2014, 2015), from three shore habitats (high shore, low shore, and tide pools) at Wairepo Reef in Kaikoura.

Data were analysed with factorial analysis of variance (ANOVA) as 3 elevations x 3 seasons x 3 locations on the host, with elevation and season as fixed factors, and location on the host being a random factor. Most test factors had high variance heterogeneity (Levene's test $p<0.01$, see Table 3.4), but no data transformation (e.g., log+1) could rectify the problem. I therefore highlight that significant effects in these tests can be associated with both different means and/or different variances

between treatments (Underwood 1997). Significant ANOVA results were followed by *post hoc* tests (LSD) to identify differences between treatments.

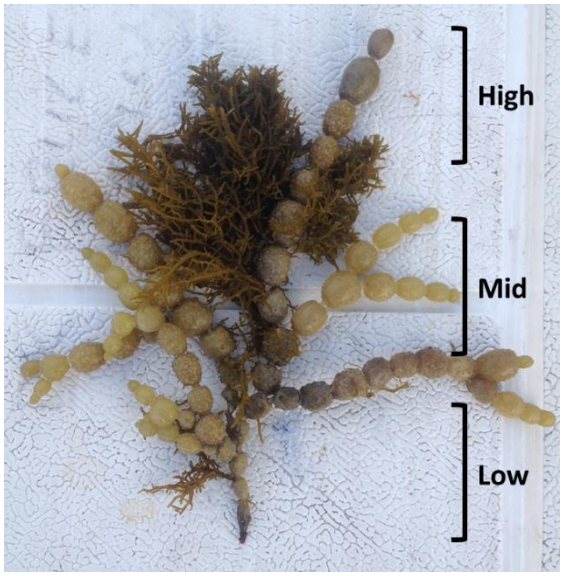

Figure 3.1. A photograph of *Hormosira banksii* depicting the low, mid, and high regions used to determine if *Notheia* attachment is linked to a particular area on its host.

Hormosira plants with *Notheia* attached were randomly collected from the low shore, high shore and tide pools (ca. 20 plants per zone, from Wairepo Reef, on 28-September 2013), to determine the sex and relative age of each host plant in the laboratory. The sex of each host plant (*Hormosira* is dioecious) was determined under a compound light microscope (100x magnification) where a *Hormosira* node was sliced transversely into thin segments, and the reproductive conceptacles were examined for male or female gametes (Figure 3.2). Each individual was aged using a visual key emphasizing the colour, size, and how visible and 'bumpy' the conceptacles were (Figure 3.3). I also recorded whether the *Notheia* holdfast was attached to the internode or to the node of its host (Figure 3.3). A chi-squared test of independence was used to test if the small-scale location where *Notheia* is attached depends on *Hormosira* age or sex.

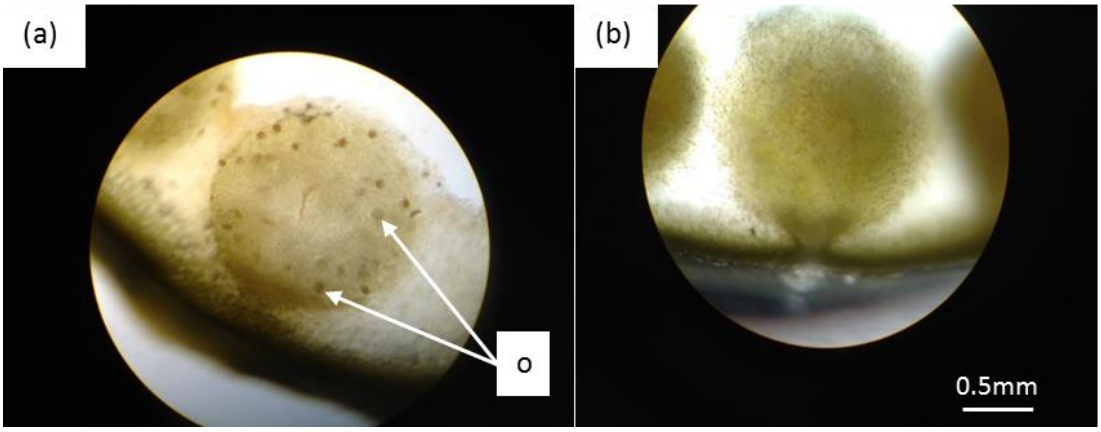

Figure 3.2. Female **(a)** and male **(b)** conceptacles of *Hormosira banksii*. A mature female conceptacle is flask-shaped and can be 0.8-1mm in width. They contain **(o)** oogonia which are 160 x 110 µm at maturity. Mature male conceptacles are similar in size, but hold numerous antheridia which are 42 x 17 µm and contain 64 sperms at maturity (Osborn 1948).

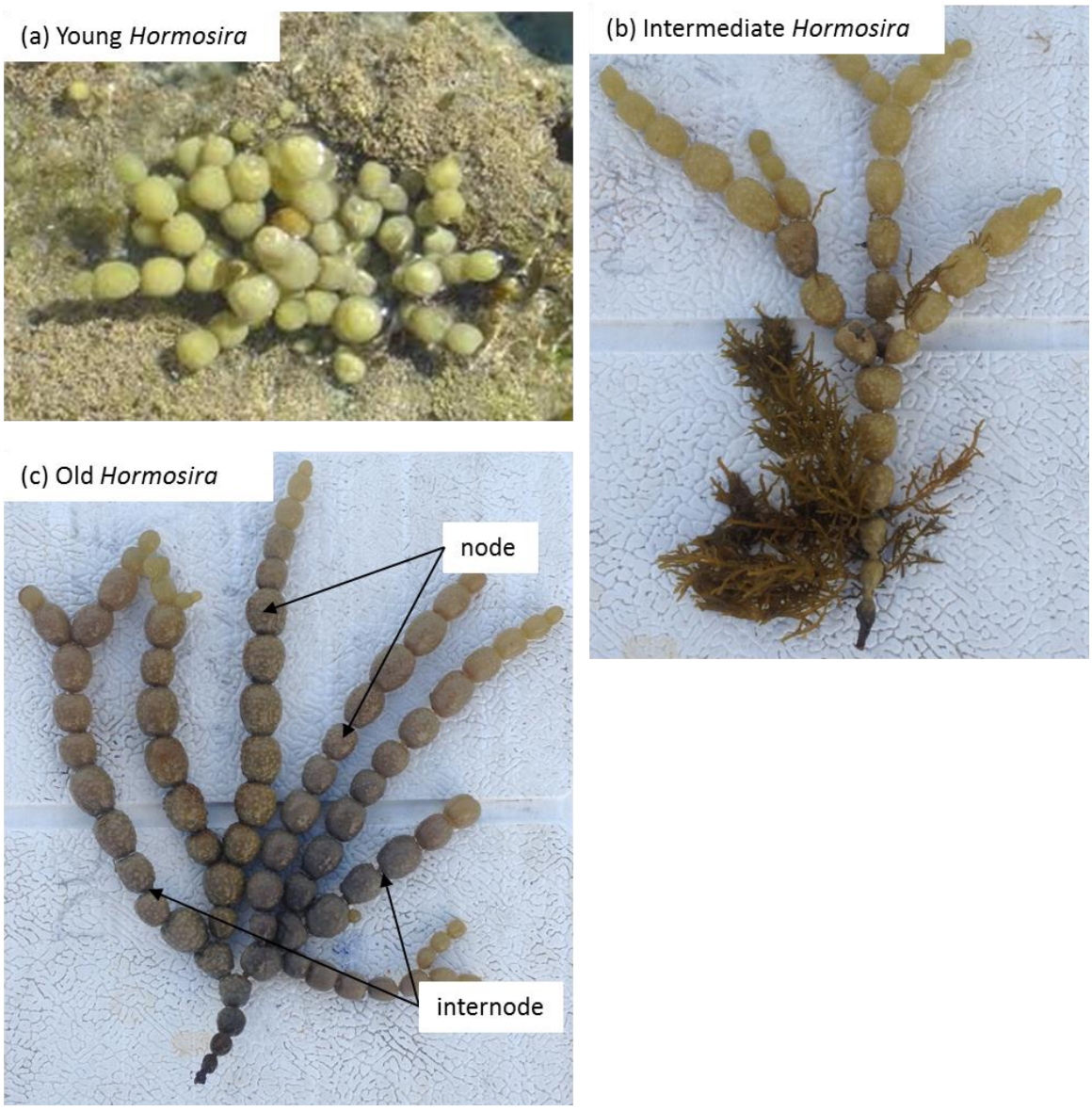

Figure 3.3. The visual key used to 'age' *Hormosira* individuals. **(a)** A young individual is light green with smooth nodes, **(c)** an old individual is dark green/grey and has very bumpy nodes displaying high fertility, **(b)** an intermediate aged individual is somewhere in between, but is easy to differentiate from both young and old.

3.3.4. Survival and growth (tagging and removal experiment)

A tagging experiment was initiated on the 5th of June 2016 to quantify changes in *Notheia* biomass over time under the 'normal' condition of being attached to its host. Twenty individuals were tagged at the low shore and in tide pools for each of four different treatments, representing different levels of hydrodynamic drag and biomass of *Notheia* (the high shore was excluded because *Notheia* only occurs as very small individuals in this zone, see result section). More specifically, I tagged big *Notheia* clumps with >4cm long fronds (high drag, high holdfast density), similar big clumps which

I trimmed back (with scissors) to ca. 0.5 cm long fronds (low drag, high holdfast density), small *Notheia* clumps (<0.5 cm; low drag, low holdfast density), and *Hormosira* beads without *Notheia* (control, to measure survival of the host itself and to document if *Notheia* recruited onto the tagged beads). Each treatment was delicately tagged with a small labelled piece of colour coded flagging tape, tied to the *Hormosira* internode one bead below where the experimental *Notheia* clump was situated (to be able to track the exact bead and *Notheia* clump over time).

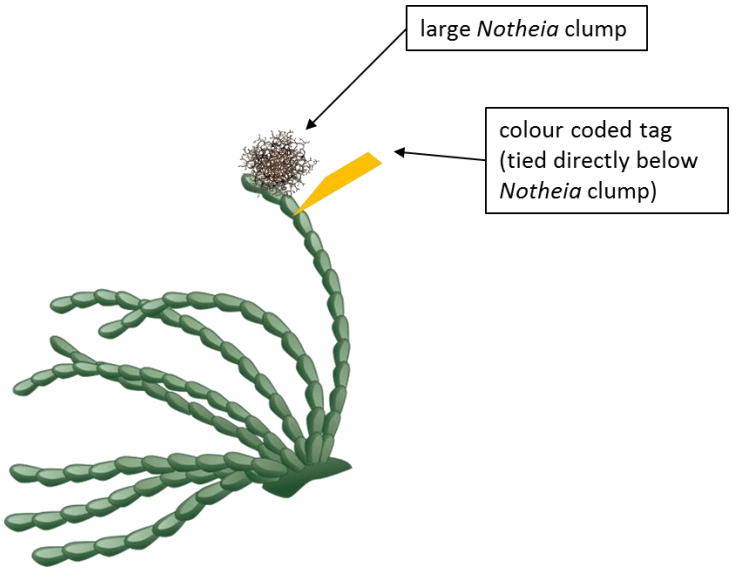

Figure 3.4. Tag position on a *Hormosira* individual in the survival and growth experiment.

The maximum width and height of each *Notheia* clump was measured to the nearest mm at the start of the experiment and monthly thereafter for 5 months, with a ruler, to allow calculation of the 'growth' (change in width x length) of each clump over time.

An additional 40 tags were added in September 2016. But due to a dramatic 7.8 magnitude earthquake on 14/11 2016, all major *Hormosira* beds and *Notheia* (including my experiment) along ca. 120 km coastline was destroyed, and therefore these extra tags were measured only once during initial setup and once in October.

Many tags were lost over 5 months among all treatments, so to simplify the analysis and better test if and how drag and holdfast density affects survival and growth, data collected at the last sampling event (13-October) were excluded from the statistical analysis (Table 3.5). A factorial ANOVA tested

the effect of elevation (low, tide pools) and treatment on percent change in *Notheia* biomass from the start of the experiment to 4 months. Both factors (elevation and treatment) had homogenous variances (Levene's test $p>0.05$, Table 3.6) and the analysis was therefore performed on untransformed data.

3.3.5. Stress assays (translocation experiments)

It is possible that host specificity is only a prerequisite for the initial settlement and growth of *Notheia*, so the next set of experiments were done to test if *Notheia* could survive and grow under varying environmental conditions without being naturally attached to its host.

In April 2016, large *Notheia* clumps were collected from tide pools in Kaikoura and brought to the laboratory. Mobile invertebrates were gently washed off with running seawater, and *Notheia* was trimmed to ca. 9.5 cm^2. This area was calculated by taking a perpendicular photo of the seaweed, flattened onto a white scaled plastic sheet. Individual clumps were randomly assigned one of 14 'environmental condition' treatments (see Table 3.1 for details, including replication levels), before being transferred back into the field in a bucket with seawater.

To test for elevation **(Assay 1)**, clumps were allocated to one of three tidal elevations (high shore, low shore, tide pools), and were tied with a twist tie to a *Hormosira* host.

To test for elevation, host, and the effect of mesh bags **(Assay 2)**, clumps were allocated to either the high shore or low shore, to a *Hormosira* host or a peg drilled into bare rock, and were tied with a twist tie or placed into a mesh bag (black, with 1 mm holes to allow flow of water and nutrients).

To test for host and mesh bags **(Assay 3)**, clumps were all placed in the low shore, with either a *Hormosira*, *Cystophora* (a co-occurring alga), or bare rock host, and were tied with a twist tie or placed into a mesh bag.

Lastly, to test for nutrients and location in canopy **(Assay 4)**, clumps were all placed in tide pools on a *Hormosira* host, with ± nutrients (nutrient levels were elevated as in (Pedersen and Borum 1996, 1997, Thomsen et al. 2007) by adding two Jobes fertilizer spikes to small mesh bags), and were tied with twist ties at the base of the canopy or at the top of the canopy.

The experiment ran for 10 days, which was adequate time to estimate growth rates for fucoids (Pedersen and Borum 1996), and to detect environmental stress effects (Thomsen et al. 2007). After 10 days, the *Notheia* clumps were carefully detached from their host and brought back to the

laboratory to be photographed again. All photos were analysed using Adobe Photoshop CC 2015 to calculate the surface area of the seaweed clump in cm^2. The before and after surface area measurements were compared to calculate percent growth (positive values), percent loss (negative values), or 'death' (100% loss).

First I tested for effect of elevation by comparing treatment 2, 6, and 11. Second, I tested for interactive effects between elevation (low, high), substrate type (rock, *Hormosira*) and attachment type (mesh open) by comparing treatment 1-8. Third, I tested, at the low shore only, if *Cystophora* is of similar importance as *Hormosira* in modifying environmental conditions by comparing treatment 5-10. Finally, I tested for interactive effects of nutrients and host height by comparing treatment 11-14 (see Table 3.1 for treatment details). ANOVA was used in all analyses.

Table 3.1. Table showing 14 treatments (Trt.) used in the stress assay experiments. Treatments varied by elevation level (Ele. **(HS)** High shore, **(LS)** Low shore, **(TP)** Tide pools), nutrient level (Nutri.), host species, attachment type (Bond), and canopy level. Treatments included in each statistical test are noted with an 'x' under the associated assay test.

Trt.	Ele.	Nutri.	Host Species	Bond	Canopy Level	N	Assay 1	Assay 2	Assay 3	Assay 4
1	HS	No	*Hormosira*	Mesh Bag	Top	2		x		
2	HS	No	*Hormosira*	Twist Tie	Top	5	x	x		
3	HS	No	Bare	Mesh Bag	Top	14		x		
4	HS	No	Bare	Twist Tie	Top	5		x		
5	LS	No	*Hormosira*	Mesh Bag	Top	4		x	x	
6	LS	No	*Hormosira*	Twist Tie	Top	3	x	x	x	
7	LS	No	Bare	Mesh Bag	Top	4		x	x	
8	LS	No	Bare	Twist Tie	Top	3		x	x	
9	LS	No	*Cystophora*	Mesh Bag	Top	4			x	
10	LS	No	*Cystophora*	Twist Tie	Top	4			x	
11	TP	No	*Hormosira*	Twist Tie	Top	6	x			x
12	TP	Yes	*Hormosira*	Twist Tie	Top	6				x
13	TP	No	*Hormosira*	Twist Tie	Base	5				x
14	TP	Yes	*Hormosira*	Twist Tie	Base	4				x

Significant ANOVA results were followed by *post hoc* tests (LSD) (for tests with more than two treatment levels). Most factors had homogenous variances or low variance homogeneity (Levene's test $p>0.05$, Table 3.7), and I therefore analysed untransformed data thereby simplifying my interpretations of significant interaction effects (Quinn and Keough 2002). Within tests, some factors may have had Levene's p-values lower than 0.05, but these data could not be transformed to achieve variance homogeneity, and I therefore still analysed experiments with untransformed data (Underwood 1997).

All statistical analyses were carried out in RStudio version 0.99.892.

3.4. Results

3.4.1. Global Distribution

The herbarium data documented a distribution of *Hormosira* in Australia from Albany in Western Australia, along most of the south coast, Tasmania, and to the Sunshine Coast (at -26.04, 153.07). In New Zealand *Hormosira* has been collected along most of the coastline from the tip of the North Island to Stewart Island. *Hormosira* has also been collected at the Chatman Islands, but not from any of the sub-Antarctic islands. *Notheia* has a similar collection pattern, although it has not been collected from the northern range of *Hormosira's* distribution in east Australia or from most of the east coast of the North Island, and most of the west coast of the South Island in New Zealand.

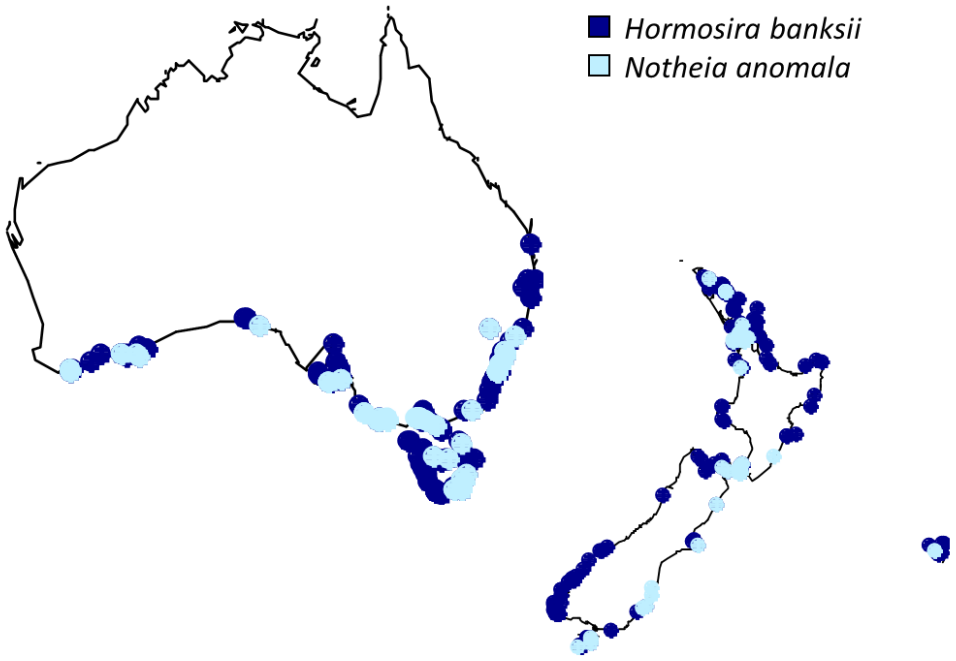

Figure 3.5. A map of Australia (left) and New Zealand (right) with dark and light blue data points showing position of herbarium collections, for *Hormosira* and *Notheia*, respectively.

3.4.2. Distribution and abundance on vertical, regional, and temporal scales

There were no significant interactions between elevation and region for *Hormosira* or *Notheia* (Table 3.2). However, both *Hormosira* and *Notheia* were strongly affected by elevation (Table 3.2), but with contrasting patterns; *Hormosira* was least and *Notheia* was most abundant in tide pools (Figure 3.6). There were higher abundances of *Hormosira* at Kaikoura than Moeraki, a pattern that appeared to be due to higher abundances at low elevation and in tide pools (Figure 3.6).

Table 3.2. ANOVA results testing the distribution of *Hormosira banksii* and *Notheia anomala* at three tidal elevations and two sampling regions (Kaikoura and Moeraki) during summer. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity are shown in brackets following each factor.

Test	Treatment	SS	Df	F-Value	P-Value
Hormosira	Elevation [0.164]	21938	2	14.623	**<0.001**
	Region [0.263]	7830	1	10.438	**0.002**
	Elevation x Region	2994	2	1.996	0.146
	Error	40504	54		
Notheia	Elevation [0.002]	73.67	2	7.505	**0.001**
	Region [0.1]	15.81	1	3.222	0.078
	Elevation x Region	23.42	2	2.386	0.102
	Error	265.02	54		

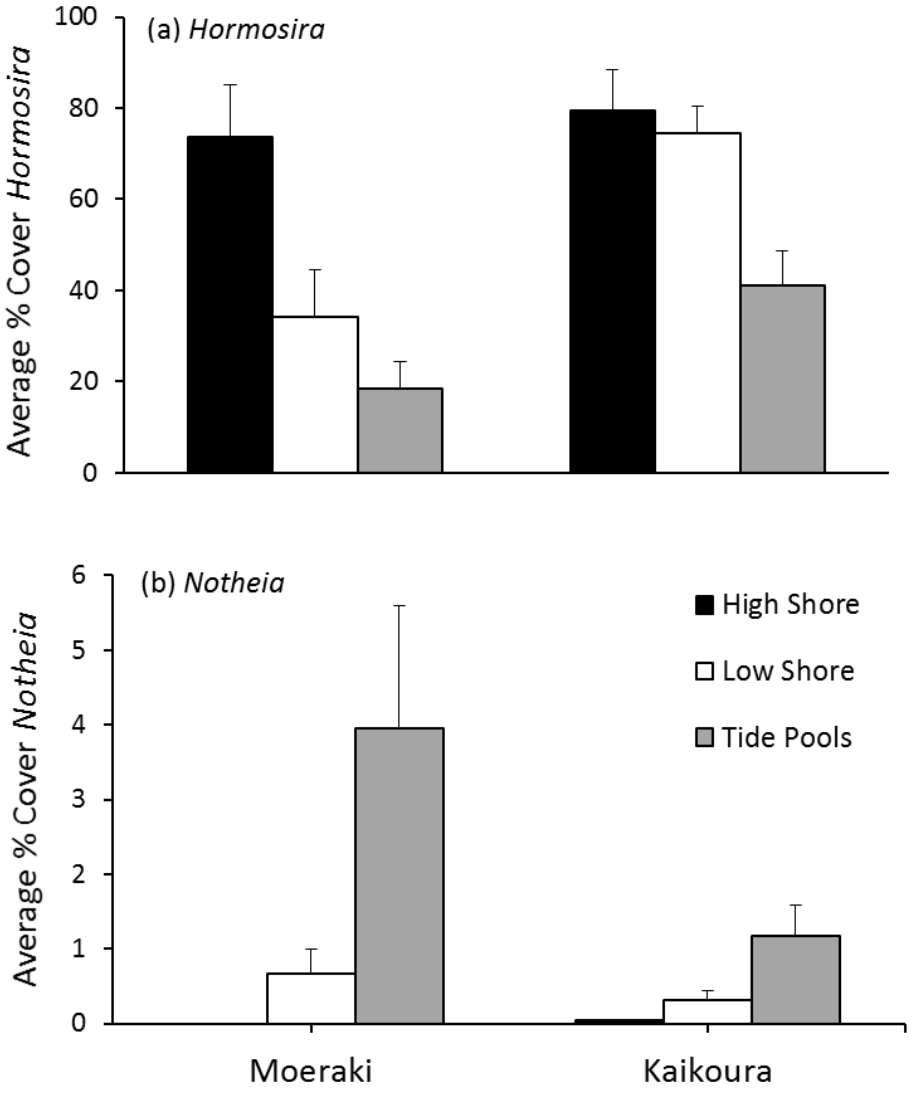

Figure 3.6. Percent cover of *Hormosira* (a) and *Notheia* (b) at three elevation levels during Summer at Moeraki Peninsula and Wairepo Reef, Kaikoura. n= 10 for each elevation; error bars are standard error.

There was a significant interaction between elevation and season on the abundance of *Hormosira* (Table 3.3), with summer being the only season where abundance was greater in the high shore than the low shore and tide pools (Figure 3.7a, Table 3.3). Note however, the SS and F values were an order of magnitude larger for the single factor "elevation" than the interaction term (Table 3.3). There was a significant effect of elevation on the cover of *Notheia* due to higher abundances in tide pools, compared to the low and high shore elevations ($p<0.001$) (Figure 3.7b).

Table 3.3. ANOVA results testing the abundance of *Hormosira banksii* and *Notheia anomala* at three tidal elevations and four seasons at Kaikoura. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity is shown in brackets following each factor.

Test	Treatment	SS	Df	F-Value	P-Value
Hormosira	Elevation [0.112]	101488	2	78.146	**<0.001**
	Season [0.805]	688	3	0.353	0.787
	Elevation x Season	8567	6	2.199	**0.045**
	Error	128572	198		
Notheia	Elevation [<0.001]	276.8	2	23.630	**<0.001**
	Season [0.467]	17.3	3	0.983	0.402
	Elevation x Season	47.7	6	1.358	0.234
	Error	1159.9	198		

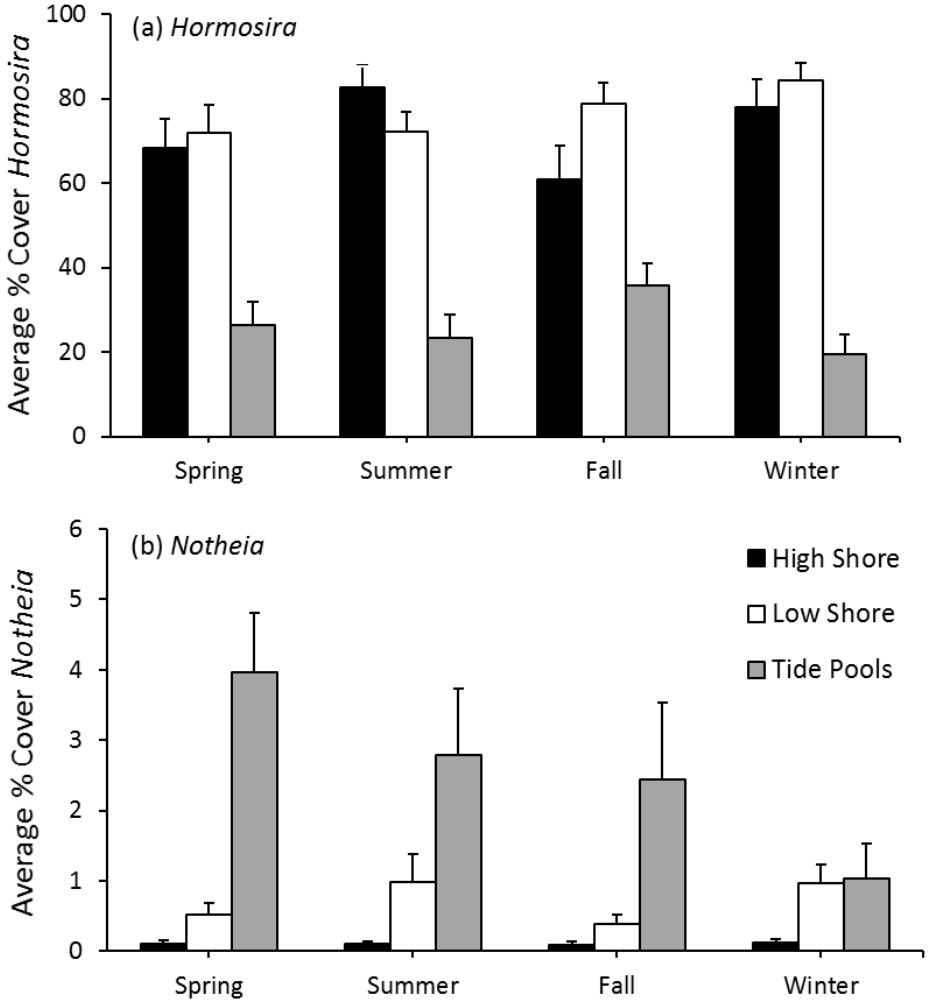

Figure 3.7. Percent cover of *Hormosira* (a) and *Notheia* (b) at three shore habitats across all four seasons. The survey values are from Kaikoura (Wairepo Reef) only. Error bars are standard error. n= 20 for Spring, Summer, and Fall, n= 10 for Winter.

3.4.3. Distribution and abundance on host plant scale (point of attachment)

There was significant variation associated with the location of *Notheia* clumps on *Hormosira*. The ANOVA showed significant season x elevation ($p<0.001$) and elevation x location interactions ($p<0.001$) (Table 3.4). Graphical analyses suggest that in fall, low shore *Hormosira* individuals have a greater number of mid and high *Notheia* attachments than the tide pools, whereas in spring and summer, the low shore and tide pools showed similar attachment patterns (Figure 3.8). The high shore individuals had few clumps growing on them, with the greatest numbers found in the low region of the host, an opposite pattern to the low shore and tide pool populations where most *Notheia* clumps were attached to the mid and high regions of the host.

The highest F and SS value was, again, for the elevation factor, demonstrating that elevation has a very strong effect on the location of *Notheia* clumps on its host (Table 3.4).

Table 3.4. ANOVA results for the effect of season, elevation, and location on host, on the number of attached *Notheia* clumps. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity are shown in brackets following each factor.

Test	Treatment	SS	Df	F-Value	P-Value
Clump Location	Season (Sea) [0.002]	39.2	2	7.193	**<0.001**
	Elevation (Ele) [<0.001]	200.5	2	36.779	**<0.001**
	Location (Loc) [<0.001]	93.7	2	17.179	**<0.001**
	Sea x Ele	51.3	4	4.708	**<0.001**
	Sea x Loc	16.4	4	1.508	0.198
	Ele x Loc	59.1	4	5.423	**<0.001**
	Sea x Ele x Loc	20.6	8	0.944	0.479
	Error	2387.7	876		

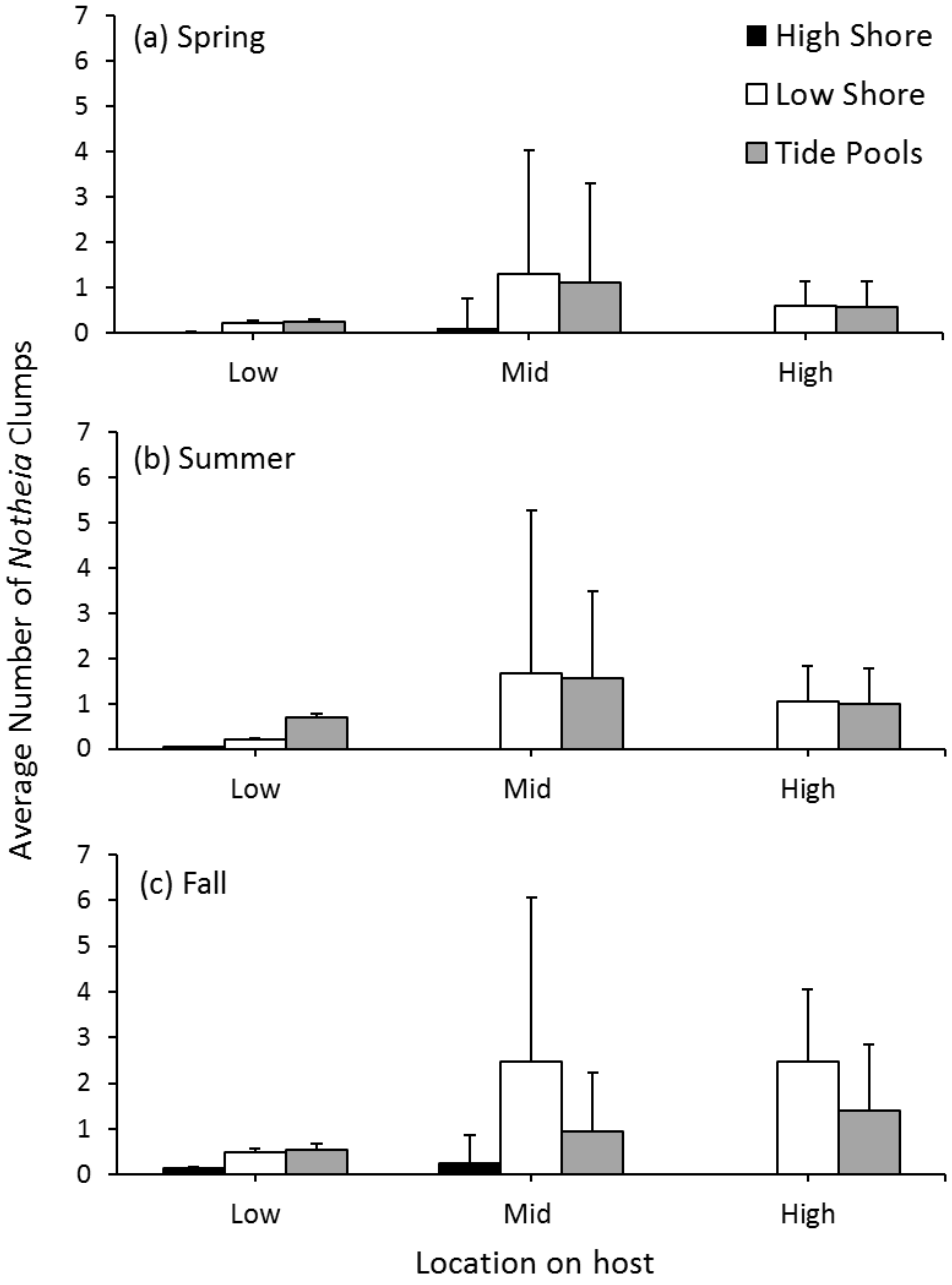

Figure 3.8. The average (± SE) number of *Notheia* clumps found in the low, mid, and high region of a *Hormosira* host in the high shore, low shore, and tide pools. Seasonal variations are shown for spring (a, n=120), summer (b, n=210), and fall (c, n=60).

Out of 75 collected epiphytised *Hormosira* plants, 35 were males and 40 were females, and a chi-square test showed that this ratio was not different from 1:1, suggesting that *Notheia* does not have preference towards either male or female hosts. Furthermore, a chi-square test of independence determined that the point of attachment was not independent of host age ($p=0.01$, $df=2$, $X^2=8$), with more *Notheia* attached on *Hormosira* nodes than internodes, but only for intermediate and old hosts

(Figure 3.9). Personal observations highlighted that attachment was not always directly connected to a *Hormosira* conceptacle (as can be seen in Figure 3.10).

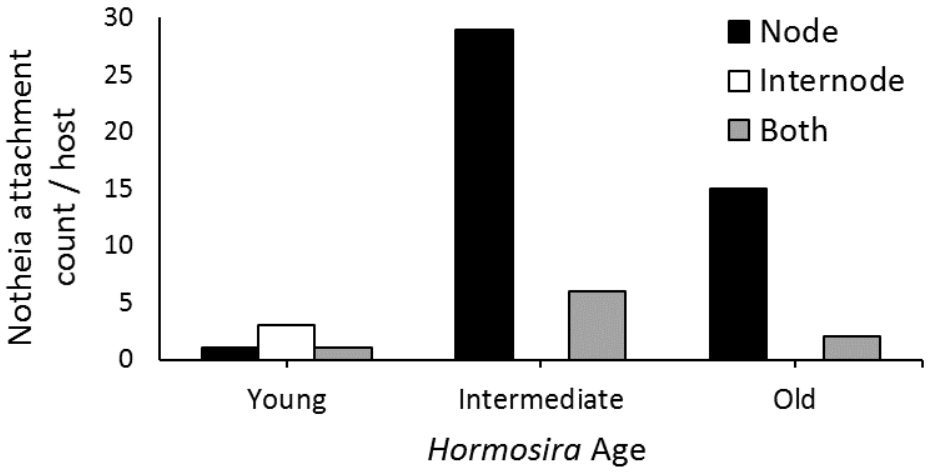

Figure 3.9. The attachment point of *Notheia* is not independent of *Hormosira* age. Intermediate and old hosts hold greater *Notheia* attachments. Samples collected from Wairepo Reef.

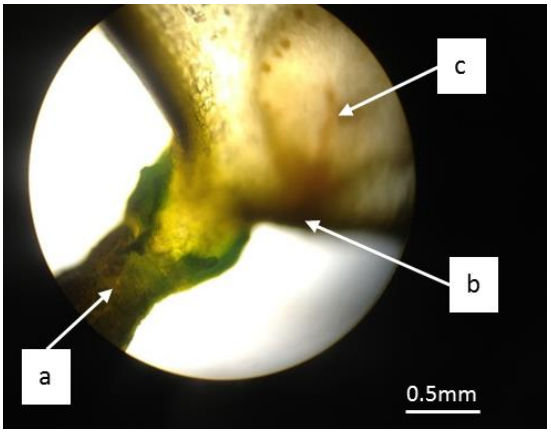

Figure 3.10. The basal **(a)** holdfast of *Notheia* is not attached directly to a female *Hormosira* **(b)** ostiole. A female *Hormosira* **(c)** conceptacle is also depicted. 100x magnification.

3.4.4. Survival and growth (tagging and removal experiment)

Over the course of six months there was a gradual loss of tags across all treatments and in both habitats (Table 3.5, Figure 3.11). The pattern of loss over time for each treatment does not show any differences. After five months, >70% of all tags across all treatments had been lost. I therefore excluded this sampling period from statistical analyses. All samples (tags) that were lost by 'Time 4' were also excluded from the analysis, leaving sample sizes of 8, 12, 10, and 11 in the low shore, and 10, 6, 11, and 11 for the tide pools (for big clump, big clump trimmed, small clump, control, respectively). There were no effects of treatment or habitat on change in *Notheia* biomass between 'Time 0' and 'Time 4' (Table 3.6).

Table 3.5. The average percent of tags lost over time for both habitats and all treatments. By 'Time 5' >70% of tags had been lost, resulting in the exclusion of this sampling period from the analysis.

Habitat	Treatment	Time 0	Time 1	Time 2	Time 3	Time 4	Time 5
Low Shore	Big Clump	0	10	35	60	65	70
	Big Clump Trimmed	0	5	35	45	55	70
	Small Clump	0	0	15	35	45	80
	Control	0	0	30	45	55	100
Tide Pools	Big Clump	0	30	35	45	50	80
	Big Clump Trimmed	0	20	40	65	70	90
	Small Clump	0	10	10	25	45	75
	Control	0	10	15	40	45	90

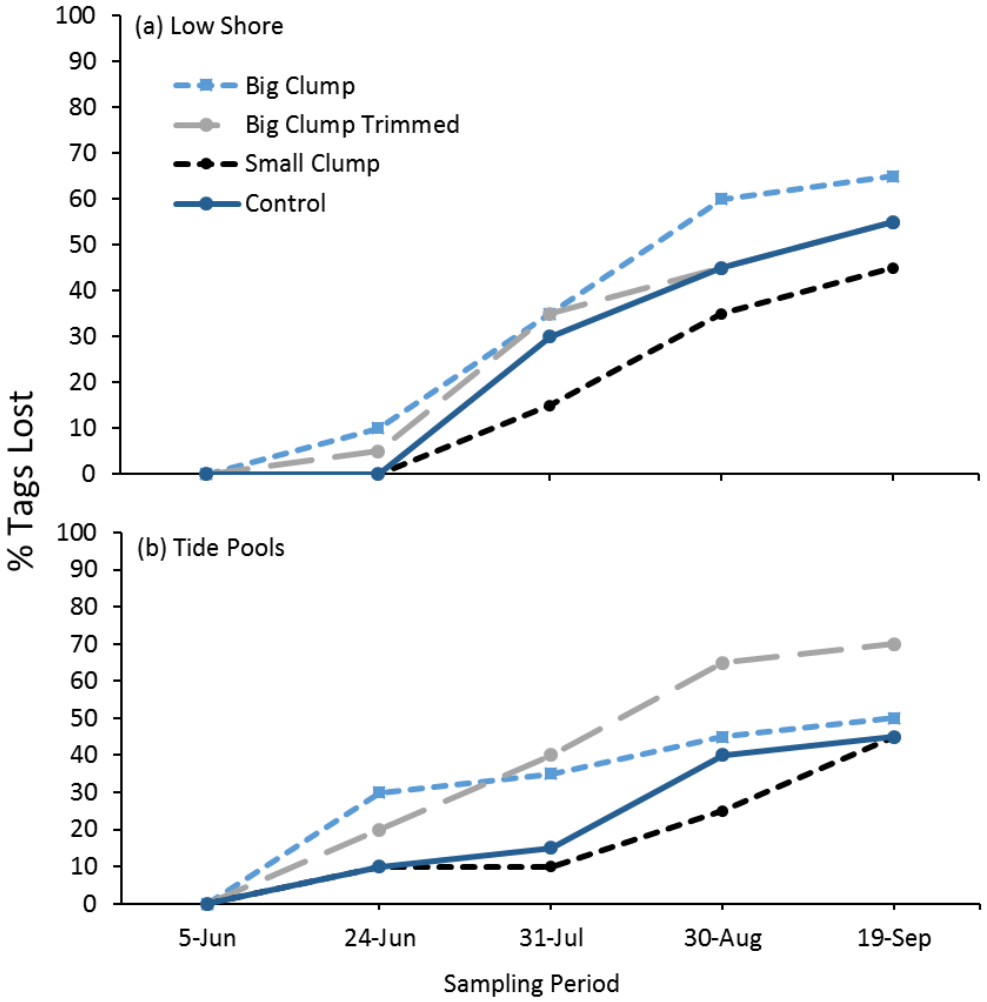

Figure 3.11. Proportion of samples lost over time in the low shore (a) and tide pool (b) tagging experiment.

Table 3.6. ANOVA results when testing the effect of habitat and treatment on change in *Notheia* biomass between 'Time 0' and 'Time 4'. Levene's test for variance homogeneity is shown in brackets following each factor.

Test	Treatment	SS	Df	F-Value	P-Value
% Change in *Notheia*	Treatment [0.376]	38896	1	0.667	0.417
	Habitat [0.439]	77235	3	0.441	0.724
	Treatment x Habitat	291246	3	1.665	0.182
	Error	4140873	71		

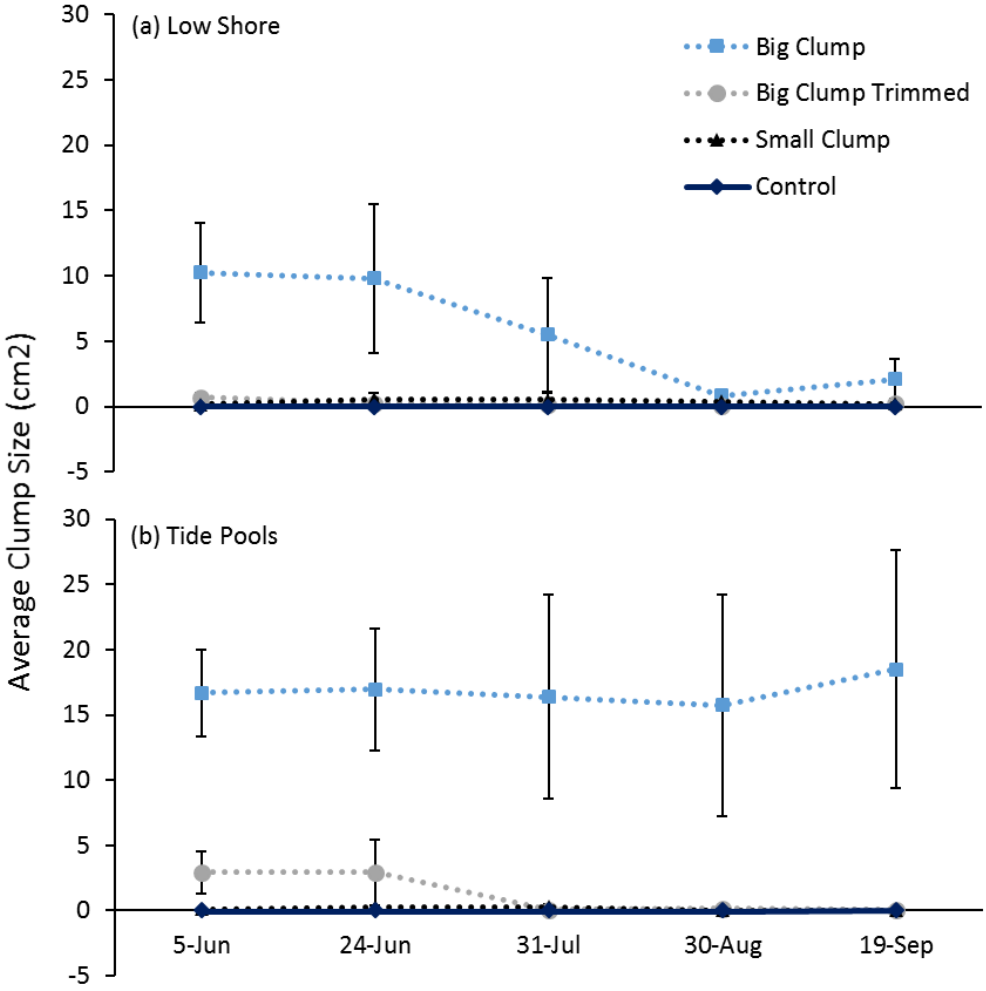

Figure 3.12. *Notheia* clumps over time in the low shore (a) and tide pools (b). Error bars are standard error.

3.4.5. Stress assays (translocation experiments)

In the first stress assay there was a significant effect of elevation, demonstrating that biomass loss was higher in the high shore (often with 100% loss) compared to the low shore or tide pools (Table 3.7, Figure 3.13, Figure 3.14).

In the second assay, which tested for elevation, host, and mesh bags, there was a significant interaction effect of elevation x host, and host x mesh bag. There were much stronger negative effects of the bare rock 'host' on *Notheia* biomass in the high shore than in the low shore, and *Notheia* in mesh bags tied to a *Hormosira* host (in the high shore) had significantly more loss than *Notheia* in mesh bags tied to bare rock (Figure 3.13). Overall, the highest SS and F-values were for the elevation and host factors (Table 3.7).

The third stress assay from the low shore experiment showed a significant effect of host substrate, mesh bags, with a strong host x mesh bag interaction effect (Table 3.7). In all host treatments (bare rock, *Cystophora* host, *Hormosira* host) *Notheia* growth showed a positive percent change when the mesh bag was used (Figure 3.13). However, the *Hormosira* host treatment had high variability in biomass loss when enclosed in a mesh bag, resulting in the interaction effect. *Notheia* biomass loss was greatest when *Notheia* was attached directly to bare rock with no mesh bag.

Lastly, the fourth assay from the tide pool experiment demonstrated that there was no effect of either nutrient addition or location in the *Hormosira* canopy on *Notheia* growth (Table 3.7, Figure 3.14).

Table 3.7. ANOVA results from the stress assay tests depicted in Table 3.1. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity are shown in brackets following each test factor.

Test	Treatment	SS	Df	F-Value	P-Value
Assay 1	Elevation (Ele) [<0.001]	106.7	2	4.636	**0.016**
	Error	437.4	38		
Assay 2	Ele [<0.001]	829.1	1	17.912	**<0.001**
	Host [0.054]	920	1	19.875	**<0.001**
	Mesh Bag [0.686]	0.1	1	0.003	0.958
	Ele x Host	680.5	1	14.701	**<0.001**
	Ele x Mesh	20.1	1	0.435	0.514
	Host x Mesh	207	1	4.471	**0.042**
	Ele x Host x Mesh	173	1	3.738	0.062
	Error	1573.8	34		
Assay 3	Host Substrate [0.19]	1.813	2	6.156	**0.01**
	Mesh Bag [<0.001]	9.842	1	66.825	**<0.001**
	Host x Mesh	2.523	2	8.565	**0.003**
	Error	2.356	16		
Assay 4	Fertiliser [0.278]	0.101	1	0.296	0.593
	Location in Canopy [0.794]	0.120	1	0.353	0.560
	Fertiliser x Location	0.271	1	0.793	0.386
	Error	5.809	17		

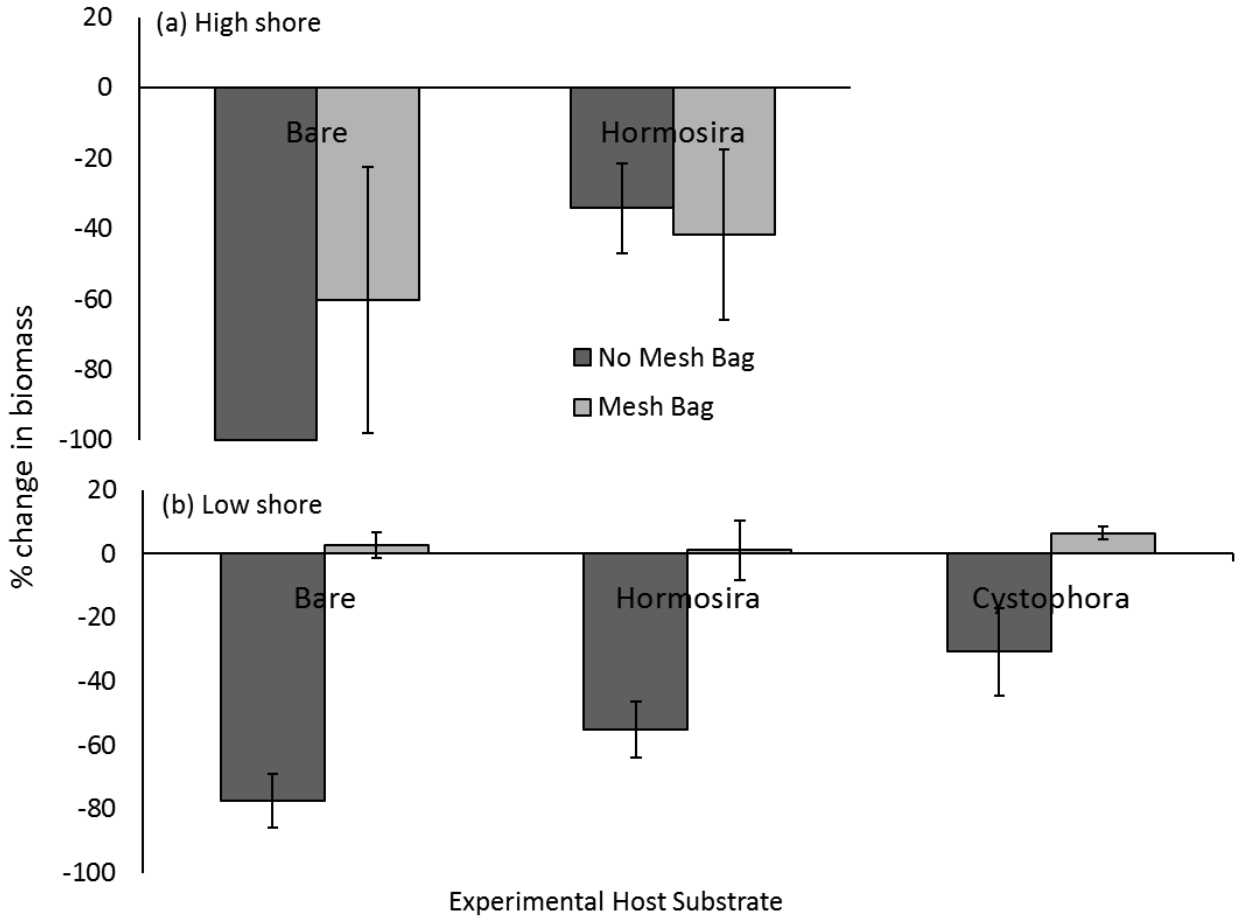

Figure 3.13. Translocation experiment performed in the high shore (a), and low shore (b) looking at the effect of host canopy and mesh bags (desiccation) on *Notheia* growth. Data set does not include samples that were lost over time. n= 5, 2, 14, 5 respectively for high shore. n= 4 for all treatments, apart from bare host with no mesh bag and *Hormosira* host with no mesh bag which are 3. Error bars are standard error.

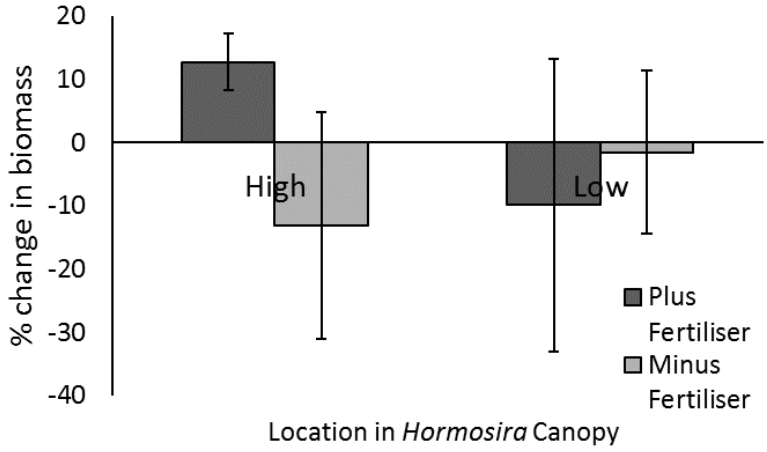

Figure 3.14. Translocation experiment performed in tide pools looking at the effect of location in canopy and increased nutrients on *Notheia* growth. Data set does not include samples that were lost over time. n= 6 for high canopy treatments, n= 5 for low canopy treatments. Error bars are standard error.

3.5. Summary

When comparing the distribution patterns of *Hormosira* and *Notheia* across Australasia I found a close relationship, with herbarium collections of *Notheia* generally closely overlapping collections of *Hormosira*. However, *Notheia* was noticeably absent from the northern limit of *Hormosira* (in eastern Australia) suggesting that *Notheia* is less tolerant to environmental stressors (such as heat stress) than its host. *Notheia* was also absent from large stretches of New Zealand's coastlines, which could be due to unsuitable habitat, or might be associated with low collection efforts rather than representing true absences.

At Moeraki and Kaikoura, there were contrasting patterns of *Hormosira* and *Notheia* distribution, with *Hormosira* being least and *Notheia* most abundant in tide pools (a distribution that has been observed in other studies (Thomsen et al. 2016)). There were also differences in canopy cover among regions. In Kaikoura, *Hormosira* was more abundant in the low shore, and *Notheia* was less abundant in the tide pools than at Moeraki. It has been shown that *Hormosira* is one of the most desiccation-resistant canopy formers in temperate Australasia, allowing it to dominate at higher elevation ranges in the intertidal zone (Schiel 2006). In comparison, I suspect that *Notheia* is more susceptible to desiccation stress, which is why it is limited to growing on *Hormosira* fronds in the wetter environments such as tide pools and the low shore (Hallam et al. 1980). When I quantified the intertidal distribution patterns of these two species over the span of a year at Wairepo Reef, I found that there were no significant differences among seasons, but contrasting elevation effects in

abundance were observed again. *Hormosira* is a perennial species (Schiel 2011), and therefore canopy cover is expected to be relatively constant across the year.

The small-scale attachment of *Notheia* varied among elevations. The greatest number of *Notheia* clumps in the high shore were in the low region of their *Hormosira* hosts. By contrast on the low shore and in tide pool *Hormosira* populations, most *Notheia* clumps were found on the mid and high regions of the host. In the high shore, *Notheia* is likely to survive better low in the canopy where moisture levels likely are greatest. Alternatively, in the low shore and tide pools, desiccation stress is lower but low-light stress higher, and it is therefore more advantageous to be attached higher up in the canopy. I found that *Notheia* was equally likely to be found attached to male and female plants of *Hormosira* (Hallam et al. 1980, Ducker and Knox 1984), and that *Notheia* holdfasts were commonly attached to other parts of *Hormosira* than its ostiole. It therefore appears that the obligate dependency is not a direct physical link to its host's reproductive structures. *Notheia* fronds were found more frequently on intermediate aged *Hormosira* plants rather than the younger ones. It could be that as *Hormosira* gets older there is a degenerative effect on the protective functions that are put in place to prevent epiphytic attachment (Durante and Chia 1991, Jennings and Steinberg 1997). In young *Hormosira* individuals, *Notheia* attachment was more frequently found on the internodes, which could be further evidence suggesting that younger individuals have higher defences to prevent epibiosis near the reproductive structures. However, it could be that there simply isn't enough surface area or depth for a *Notheia* holdfast to anchor itself into the receptacular tissue of these younger plants (Wahl 1989, Thornber et al. 2016).

Notheia individuals in the low shore and tide pools experienced high mortality due to high *Hormosira* dislodgement rates during the winter months of 2016. However, change in *Notheia* biomass was not significantly due to experimental density treatments during the first four months of the experiment. This suggests that growth in this species may not density-dependent, unlike other species such as the green alga *Ulva* (Viaroli et al. 1996). In most treatments, *Notheia* biomass reduced significantly, and especially in 'low density' treatments the individual often disappeared entirely. *Notheia* populations are likely to be very resilient, as *Notheia* has consistent biomass throughout the year (Figure 3.7). Perhaps if this experiment was run in summer the results would show more growth and less *Hormosira* dislodgement due to less storm events.

Results from the translocation experiment described some physical aspects driving *Notheia* distribution in the intertidal zone. Low shore *Notheia* individuals translocated to the high shore had high mortality rates. These overall findings were to be expected, as natural *Notheia* populations in

the high shore represent only a fraction of the whole population in the intertidal zone, and therefore elevation is a strong factor affecting *Notheia* distribution. The low shore experiment showed that *Notheia* growth was still possible even if detached from its obligate host and artificially tied to another seaweed or to bare rock. This suggests that *Hormosira* is needed for initial settlement, but once big enough survival can be independent. Once detached there was no evidence to suggest that *Notheia* could reattach to a substrate (but this was not scientifically tested). In the field I regularly saw *Notheia* attached to *Hormosira* beads higher in the canopy, and hypothesised that these individuals have faster growth rates, however, growth rates were not affected by location in the canopy, or by added nutrients.

By testing factors that affect distribution patterns of *Notheia* on a regional, regional, and vertical scale, we are better able to understand the fundamental drivers of the life strategy of epiphytism. This information can then be used by ecologists and population biologists when assessing ecosystem stability and diversity over time, as epiphytes are key providers of food and refuge in many habitats worldwide (Bologna and Heck 1999, Stuntz 2001, Thornber et al. 2016).

Chapter Four

Notheia as a contributor to local-scale biodiversity

4.1. Abstract

Few studies have quantified habitat cascades from rocky intertidal systems across spatio-temporal gradients, where the secondary habitat former is an obligate epiphyte, or evaluated reciprocal interactions between invertebrate inhabitants and the co-occurring habitat-formers. First, I tested if invertebrate inhabitants associated with the primary habitat-forming seaweed *Hormosira banksii* are always higher in the presence of its secondary habitat-forming obligate epiphyte *Notheia anomala* across spatio-temporal scales. *Hormosira* fronds were collected with and without *Notheia* from different tidal elevations, latitudes, and seasons. There were strong positive density-dependent effects of *Notheia* on both richness and abundance of invertebrates, regardless of the spatio-temporal context and inhabitant taxa. The strongest facilitation occurred in tide pools for gastropod and amphipod species. Second, a recolonization experiment tested whether inhabitants were more facilitated by, (1) *Notheia* than *Hormosira*, (2) increasing amounts of *Notheia*, and (3) if *Notheia* was alive compared to an abiotic mimic. Hypotheses 1 and 2 were strongly supported, but not Hypothesis 3. *Notheia* facilitated more invertebrates than *Hormosira*, and the facilitative effects grew with increasing *Notheia* biomass. But, richness and abundances of inhabitants were similar between living and mimic epiphytes, which suggests that *Notheia* is more importantly providing habitat space than a food source for these inhabitants. Finally, I hypothesized that inhabitants exert little or no grazing pressure on *Hormosira* and *Notheia.* This was tested in laboratory choice experiment focusing on potential grazing effects from herbivorous gastropods. I found negative effects of gastropods on both *Hormosira* and *Notheia*, with greater grazing on *Notheia*. However, grazing rates were low overall, indicating that under natural field conditions, other stressors such as light limitation, nutrient stress, desiccation and storms, are likely to swamp grazing effects. In support of the spatio-temporal surveys and colonization experiment, the grazing experiment also suggests that *Notheia* provides a better habitat for small grazing invertebrates than *Hormosira*. My results support a growing number of habitat cascade studies from different ecosystems, and suggest that these types of processes are common in marine benthic systems where epibiosis is a usual occurrence.

4.2. Introduction

Direct negative species interactions, such as predation and competition, are traditionally considered to be the main factors driving community dynamics (Gause 1935, Connell 1961, Paine 1966, Bomze 1983). Indirect species interactions were initially also considered to be driven by chains of negative species effects. For example, Paine in his *Pisaster* sea star removal experiments demonstrated that a predator can have indirect positive effects on inferior competitors (like barnacles), by preferentially consuming strong competitors (like mussels). However, over the last 30 years, direct positive species interactions, such as mutualism, and modification and formation of biogenic habitat have become incorporated into ecological theory. More recently, chains of positive species interactions, often referred to as habitat cascades (indirect positive effects on focal organisms mediated by successive facilitation in the form of biogenic formation or modification of habitat, (Thomsen et al. 2016)) have also been recognised to be important processes in community structures.

Habitat cascades are particularly common where primary habitat-forming species provide physical attachment space for secondary habitat-forming species, creating a positive net effect on organisms that depend on biogenic habitats (hereafter 'inhabitants') (Ellwood and Foster 2004, Gribben et al. 2009, Thomsen et al. 2010). There is a growing number of studies that have demonstrated habitat cascades in different ecosystems. For example, Ødegaard (2000) showed that beetles living on 'epiphytic' liana plants were more abundant and more specialised than beetles associated with the host tree. Similarly, Ellwood and Foster (2004) found that invertebrate biomass was two orders of magnitude higher in epiphytic ferns than in tree canopies in a Bornean rainforest, concluding that the ecological role of large epiphytes in rainforests has been dramatically underestimated.

It has been suggested that habitat cascades are important in crowded systems where 'epibiosis' is common, that is, where sessile habitat forming species can attach to other sessile habitat forming species. For example, terrestrial forests can be a 'crowded' ecosystem, where nest epiphytes, orchids, lianas, mistletoes, stranglers, ferns, lichens and fungi can grow on trees and bushes, competing for vital resources such as space and sunlight. Similar to these dense epiphyte-dominated forests, space can also be a limiting factor in shallow rocky intertidal habitats where species assemblages are also 'crowded' (Dayton 1971, Wahl 1989, Wahl and Hay 1995). It seems probably, therefore, that habitat cascades should also be common on rocky intertidal shores.

However, only a few studies have documented habitat cascades from rocky shores (Martin-Smith 1993, Viejo and Åberg 2003, Bell et al. 2014, Thomsen et al. 2016). Furthermore, most studies on habitat cascades have focused on epiphytes with low host specificities (Hall and Bell 1988, Edgar and Robertson 1992, Martin-Smith 1993), or sampled from a few environments, places, or temporal events (Hallam et al. 1980, Thomsen et al. 2016). Finally, only few studies have evaluated reciprocal interactions between inhabitants and their co-occurring habitat-formers, implying that although habitat cascades have been documented in many different ecosystems and places, little is known about the underpinning processes that maintain or inhibit them.

To address these issues I first quantified mobile invertebrates associated with the common canopy forming rocky intertidal seaweed *Hormosira banksii* and its obligate epiphyte *Notheia anomala* at different elevations, latitudes and seasons. Several studies have shown that *Hormosira* itself facilitates a suite of invertebrates and nursery fish, with experimental removal resulting in immediate loss of almost the entire epifaunal community (Underwood 1999, Lilley and Schiel 2006) (Figure 4.1). However, less is known about the effect of *Notheia,* which has a very different finely branched morphology (and therefore increased surface area) compared to *Hormosira*. Although, it has been shown that *Notheia* can increase the diversity of inhabitants, this single study was only conducted at one site over two sample events and did not account for the biomass of *Notheia* in the biodiversity analysis thereby confounding species identity and biomass effects (Thomsen et al. 2010, Thomsen et al. 2016). In my survey I tested the findings of Thomsen et al. (2016) in more detail and, by standardizing inhabitant data per unit of biomass, was able to evaluate if *Notheia* is a better habitat than *Hormosira*. I hypothesised that invertebrate diversity would be higher in samples where *Notheia* biomass is higher, and that this pattern would be consistent across regions and seasons.

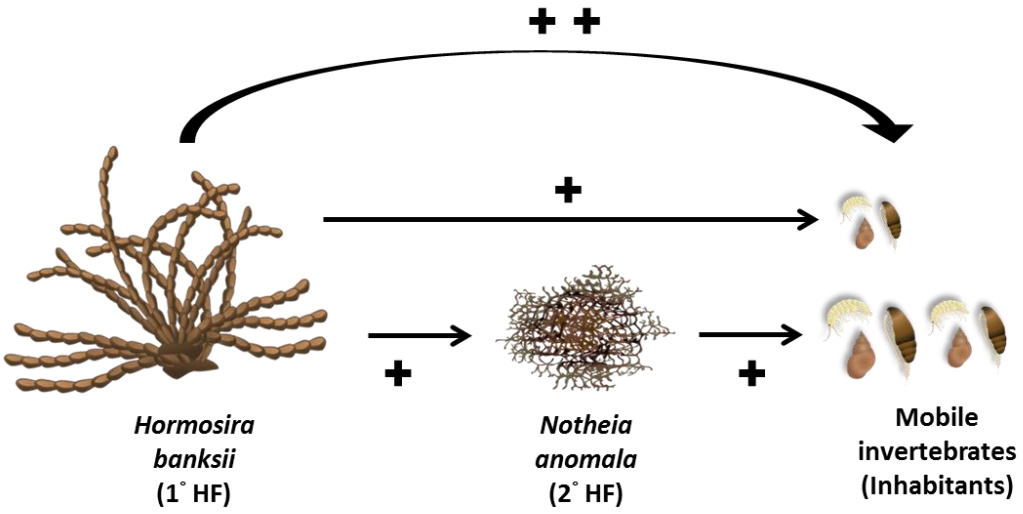

Figure 4.1. Model showing habitat facilitation starting with the **(1°HF)** primary habitat former *Hormosira banksii*. *Hormosira* facilitates the **(2°HF)** secondary habitat former *Notheia anomala*, which could further increase the diversity of invertebrates (inhabitants). In a habitat cascade, *Hormosira* has indirect positive effects on the mobile invertebrates.

It is also important to understand the causal mechanisms that underpin habitat cascades where they exist. A key gap in past research is analysis of where and how secondary habitat formers are form-functionally 'different' to primary habitat formers and how inhabitants use the different co-existing habitat-formers.

I first tested, in a recolonization experiment, if inhabitants were more facilitated (i) by *Notheia* than *Hormosira*, (ii) by increasing amounts of *Notheia* and (iii) if *Notheia* was alive compared to an abiotic mimic. I hypothesised that *Notheia* is providing a more structured habitat for invertebrates to take refuge, and that it is providing an abundant food source. I also hypothesised that there is a biomass threshold effect, so that more inhabitants are facilitated in high than low epiphyte treatments (after taking into account their higher biomass).

Finally, I tested, if herbivorous gastropod inhabitants affect the biomass of the co-existing habitat-formers, in both a choice and no-choice laboratory experiment. Here, I hypothesised that gastropod grazers would consume more *Notheia* than *Hormosira*, with a preference for *Notheia* if given a choice.

4.3. Methods

4.3.1. Spatio-temporal distribution of mobile invertebrates

I first collected *Hormosira* fronds with and without epiphytic *Notheia* attached from three elevation levels (a 'high' zone (between 0.9 and 1.1 m above the lowest astronomical tide (LAT)), 'low' zone (between 0 and 0.5 m above LAT), and interspersed submerged rock pools (hereafter tide pools)), and from four sites at different latitudes; (1) Cape Campbell (-41.728981, 174.271525), (2) Kaikoura (-42.420553, 173.710327), (3) Banks Peninsula (-43.733446, 172.845579), and (4) Moeraki (-45.395288, 170.868468). These variations in elevation and latitude represent gradients in desiccation and temperature. I collected a minimum of three *Hormosira* fronds (epiphytised and un-epiphytised), separated by at least 1 m. Cape Campbell was sampled in winter 2015, Banks Peninsula was sampled in fall 2016, and Moeraki was sampled in summer 2013. Kaikoura was sampled seasonally for a year (2014 to 2015) to test if elevation-based results were consistent over time.

Each frond sample consisted of one *Hormosira* plant removed from the base of its singular holdfast. Fronds were collected with a swift action and immediately placed into plastic zip lock bags (to avoid mobile invertebrates escaping) (Martin-Smith 1993, Viejo and Åberg 2003, Thomsen et al. 2016). In the laboratory, invertebrates were washed onto a 250 µm sieve. Invertebrates were stored in 70% ethanol. *Notheia* was separated from *Hormosira,* before measuring the dry weights of both the host and epiphyte to nearest mg (after drying at 55 °C until no further loss could be detected). Invertebrates were counted under a dissecting microscope (40x magnification) and identified into operational taxonomic units (OTU), dominated by amphipods, harpacticoid copepods, gastropods, bivalves, ostracods, and tanaids (as in Hall and Bell (1988), Martin-Smith (1993)). The invertebrates were also sorted into size classes, including >250 µm and >1 cm. Abundance data were standardized by the combined dry weight of the host and epiphyte, whereas richness data were not standardised by biomass (i.e., analysed per frond).

The spatial survey was analysed using a factorial analysis of variance (ANOVA). The data were analysed with a model that included 2 epiphyte levels (±) × 3 elevations × 4 regions on (i) total number of invertebrates, (ii) invertebrate richness, (iii) snail abundance, (iv) copepod

abundance, and (v) amphipod abundance. The temporal survey conducted in Kaikoura was analysed with the following factorial ANOVA model: 2 epiphyte levels (±) x 3 elevations x 4 seasons. Most test factors had homogenous variances or low variance heterogeneity (Levene's test $p>0.05$), and I therefore performed the analysis on untransformed data (ANOVA is robust to non-normality and minor heteroscedasticity for factorial designs with high sample sizes) thereby simplifying my interpretations of significant interaction effects (Underwood 1997, Quinn and Keough 2002). A few test factors had Levene's p-values lower than 0.05, a common issue when there are significant effects, but transforming the data did not rectify the problem (and it is advised not to transform count data (Underwood 1997, O'hara and Kotze 2010)). For these analyses I note that significant effects can therefore be associated with different means and/or different variances between treatments. Significant ANOVA results were followed by *post hoc* (LSD) tests to identify differences between treatments.

4.3.2. Mimic and density experiment

To test if invertebrates were more facilitated by *Notheia* than an artificial mimic epiphyte, a recolonization experiment was done at South Bay and Wairepo in Kaikoura. Wairepo is characterised by a sheltered, gently sloping rocky platform, whereas South Bay has higher wave energy levels and environmental stress can be greater. *Hormosira* fronds (~ 3.71 g dry weight (DW)) were incubated in the field with five different types of epiphytism: a control (no epiphyte), a low level of *Notheia* (~ 0.39 g DW), a high level of *Notheia* (~ 1.06 g DW), a low level of a *Notheia* mimic (~ 0.69 g DW), and a high level of a *Notheia* mimic (~ 1.69 g DW). The mimics were made from plastic tuffies (see Figure 4.2 for example) with approximately similar surface to DW ratios to *Notheia*. Epiphytes were attached with a small piece of twine. Seaweeds were gently washed with filtered seawater to remove mobile invertebrates before being carefully tied to a chain (Figure 4.2). Four chains were laid out in each site (placed randomly in tide pools), with four replicates for each treatment randomly attached.

After 15 days, all fronds were collected as in the spatio-temporal survey (on page 63). Invertebrates were sorted, counted and identified as described for the survey, with the following exceptions: (i) gastropods were identified into separate 'morpho-types' (distinct morphologies based on shape, colour, and markings (Figure 4.3), as detailed taxonomic keys that define species do not exist, or are too complex for many of these micro-gastropods) and (ii) gastropods

were sorted into three size classes, including 250-500 µm, 500-1000 µm and >1 cm. *Hormosira* and epiphytes were also separated and quantified as described for the survey (on page 63).

Data were standardized and analysed, with factorial ANOVA to test the effects of epiphyte level (low/high), epiphyte type (live/artificial mimic) and sites (Wairepo/South Bay) on invertebrate richness and biomass corrected abundances of all invertebrates, gastropods, copepods, and amphipods. Most factors had homogenous variances or low variance heterogeneity (Levene's test $p>0.05$). Therefore, the ANOVA was performed on untransformed data to simplify interpretations of significant interaction effects (Underwood 1997, O'hara and Kotze 2010). Significant results were followed by LSD *post hoc* tests to identify treatment differences.

Figure 4.2. A photo showing high (left) and low (right) density artificial epiphytes (tuffies) tied to a *Hormosira* host and attached to chains in a tide pool.

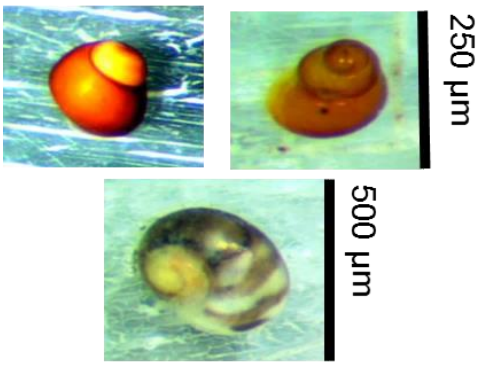

Figure 4.3. Three gastropod 'morpho-types' from Wairepo and South Bay. There were a total of 21 morpho-types. Photo credit, Alfonso Siciliano.

4.3.3. Snail grazing experiment

To test for the grazing pressure that gastropods exert on *Hormosira* and *Notheia*, a grazing experiment was conducted in the laboratory. Epiphytised *Hormosira* plants were collected from Kaikoura and brought back to the laboratory. The seaweed was rinsed with seawater into a 500 µm sieve to collect snails. To reduce stress, the seaweeds were placed in a large aerated aquarium, under halogen lights (ca. 2000 lux) set at a 12:12 LD cycle. Snails were separated from debris and other inhabitants by first decanting away positively buoyant particles, and then manually removing snails under an illuminated magnifying glass. The snails, representing the typical gastropod inhabitant community associated with *Hormosira* and *Notheia* were transferred to an aerated aquarium enclosed with a fine mesh. The snails were starved and acclimated to 18°C for one day in a temperature controlled room (in a 12:12 LD cycle). Pilot experiments had shown that the snails survive and graze seaweeds under these conditions.

To test for a grazing effect, 24 small containers (150 mL) were set up, in the following design: 2 snail levels (± 'a 1/4 teaspoon' corresponding to ca. 200 snails) × 2 food choices (*Hormosira* vs. *Notheia*) x 2 experimental conditions (*Hormosira* and *Notheia* added in mono or mixed cultures) x 3 replicates.

Ca 0.25 g wet weight of *Hormosira* and 0.021 g of *Notheia* was added to containers. The wet weights were measured after dabbing fronds with a paper towel three times before weighing at a scale of 0.0000 g. One small *Hormosira* bead was used for the *Hormosira* (ca. 0.25 g wet weight) and a very small clipping of *Notheia* (ca. 0.02 g wet weight), in part to reduce

variability of biomass measurements, in part to increase the potential to detect a grazing effect. Mesh lids were then added to each container that were placed randomly in a tray. Three pendant light and temperature loggers were placed in three empty containers with mesh lids to ensure that the temperature and light conditions were stable. Seawater was changed daily and the temperature checked regularly to avoid overheating. At the end of the experiment, snails were carefully washed off and the biomass of the seaweeds measured again with the same procedure.

Finally, the percentage change in biomass was analysed with factorial ANOVA on the full datasets. All factors had homogenous variances ($p>0.05$ in Levene's Test, Table 4.5), and significant ANOVA results were followed by LSD *post hoc* tests.

All analyses were carried out in RStudio version 0.99.892.

4.4. Results

4.4.1. Spatio-temporal distribution of mobile invertebrates

Spatial Survey:

Epiphyte presence had a strong positive effect on a variety of invertebrate diversity metrics in all statistical tests (e.g. invertebrate richness, invertebrate abundance, snail abundance, copepod abundance, and amphipod abundance) (Table 4.1). From the ANOVA output there were a series of significant interactions. For example, there were epiphyte level x region interactions for snail, copepod, and amphipod abundance. Furthermore, there was an epiphyte level x elevation interaction for amphipod abundance, and an elevation x region, and epiphyte level x elevation x region interaction for copepod abundance. These complex interactions demonstrate that life in these intertidal algal communities are highly dynamic.

There were significant effects of elevation on all diversity metrics, as a *post hoc* test (LSD) indicated that in general the high shore has much lower abundance and richness values than the low shore and tide pool habitats. Metric values for copepods were lower in the low shore, but amphipods had the highest values in the low shore.

There were often significant differences between regions. A *post hoc* (LSD) highlighted that these site-differences were not consistent across tests. For example, for the richness test, Cape Campbell showed highest values, but for the total abundance test Kaikoura and Moeraki showed the highest values. Snail abundances were lowest at Pile Bay, whereas amphipod abundances were lowest in Moeraki.

At all four sites there was a significant positive effect of *Notheia* biomass on invertebrate richness and abundance. Relationships between biomass and diversity were stronger at sites where sample sizes were larger (Table 4.2). *Notheia* biomass had stronger effects on invertebrate diversity than *Hormosira*, despite it having orders of magnitude less biomass (as in Thomsen et al. (2016)).

Table 4.1. ANOVA results of *Notheia* epiphyte level (±), tidal elevation, and latitude on invertebrate diversity. All invertebrate abundance values are standardised by host and epiphyte dry weight. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity are shown in brackets following each test factor.

Test	Treatment	SS	Df	F-Value	P-Value
Richness	Epiphyte Level (Epi)	171.4	1	82.079	**<0.001**
	Elevation (Ele) [0.63]	14.4	2	3.456	**0.034**
	Region (Reg) [0.13]	17.1	3	2.735	**0.045**
	Epi x Ele	0.7	2	0.157	0.855
	Epi x Reg	9.7	3	1.546	0.204
	Ele x Reg	13.9	6	1.110	0.358
	Epi x Ele x Reg	10.7	4	1.284	0.278
	Error	407.1	195		
Abundance	Epi	332207	1	111.865	**<0.001**
	Ele [<0.001]	35255	2	5.936	**0.003**
	Reg [0.264]	20691	3	2.322	0.076
	Epi x Ele	13059	2	2.199	0.114
	Epi x Reg	17913	3	2.011	0.114
	Ele x Reg	15642	6	0.878	0.512
	Epi x Ele x Reg	8955	4	0.754	0.557
	Error	579093	195		

Snail Abundance	Epi	110611	1	91.676	**<0.001**
	Ele [<0.001]	9286	2	3.848	**0.023**
	Reg [0.176]	14712	3	4.065	**0.008**
	Epi x Ele	7238	2	2.999	0.052
	Epi x Reg	9728	3	2.688	**0.048**
	Ele x Reg	8425	6	1.164	0.327
	Epi x Ele x Reg	4742	4	0.983	0.418
	Error	235276	195		
Copepod Abundance	Epi	1117	1	7.322	**0.007**
	Ele [<0.001]	10154	2	33.278	**<0.001**
	Reg [<0.001]	8932	3	19.515	**<0.001**
	Epi x Ele	2228	2	7.302	**<0.001**
	Epi x Reg	456	3	0.997	0.395
	Ele x Reg	3710	6	4.053	**<0.001**
	Epi x Ele x Reg	4938	4	9.092	**<0.001**
	Error	29751	195		
Amphipod Abundance	Epi	7818	1	34.060	**<0.001**
	Ele [<0.001]	2447	2	5.331	**0.006**
	Re [0.001]	4380	3	6.361	**<0.001**
	Epi x Ele	2509	2	5.466	**0.005**
	Epi x Reg	2943	3	4.273	**0.006**
	Ele x Reg	1436	6	1.043	0.399
	Epi x Ele x Reg	866	4	0.943	0.44
	Error	44760	195		

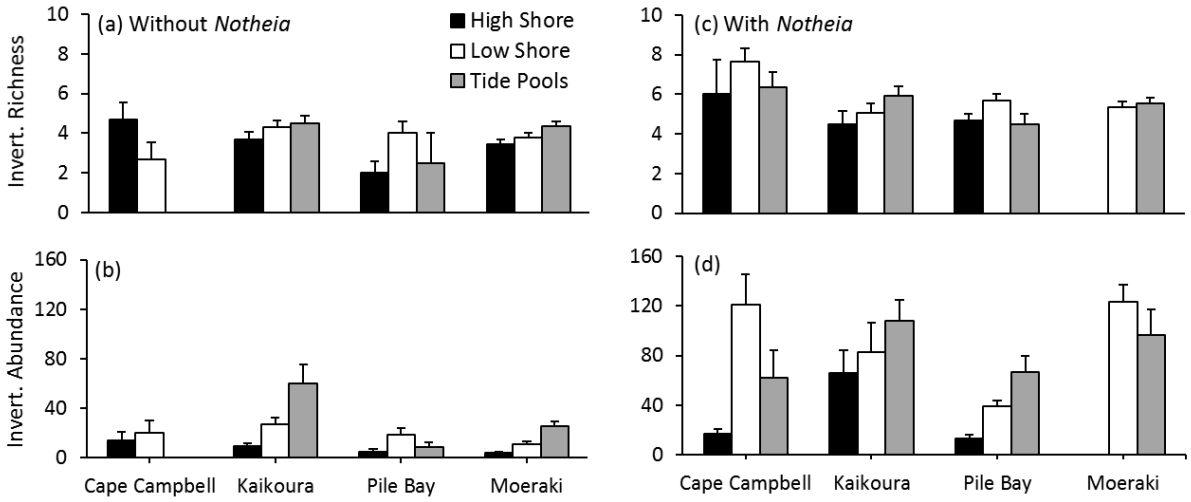

Figure 4.4. Invertebrate richness (a) and abundance (b) for *Hormosira* samples collected without *Notheia*, and invertebrate richness (c) and abundance (d) for *Hormosira* samples that had >0 g *Notheia* dry weight. The legend illustrates whether the samples were collected from the high shore, low shore, or tide pools. Essentially all invertebrates analysed were smaller than 5 mm. *Notheia* was not present in the high shore at Moeraki, which is why there are no values. Error bars are standard error. Abundance values are standardised by combined host and epiphyte dry weight.

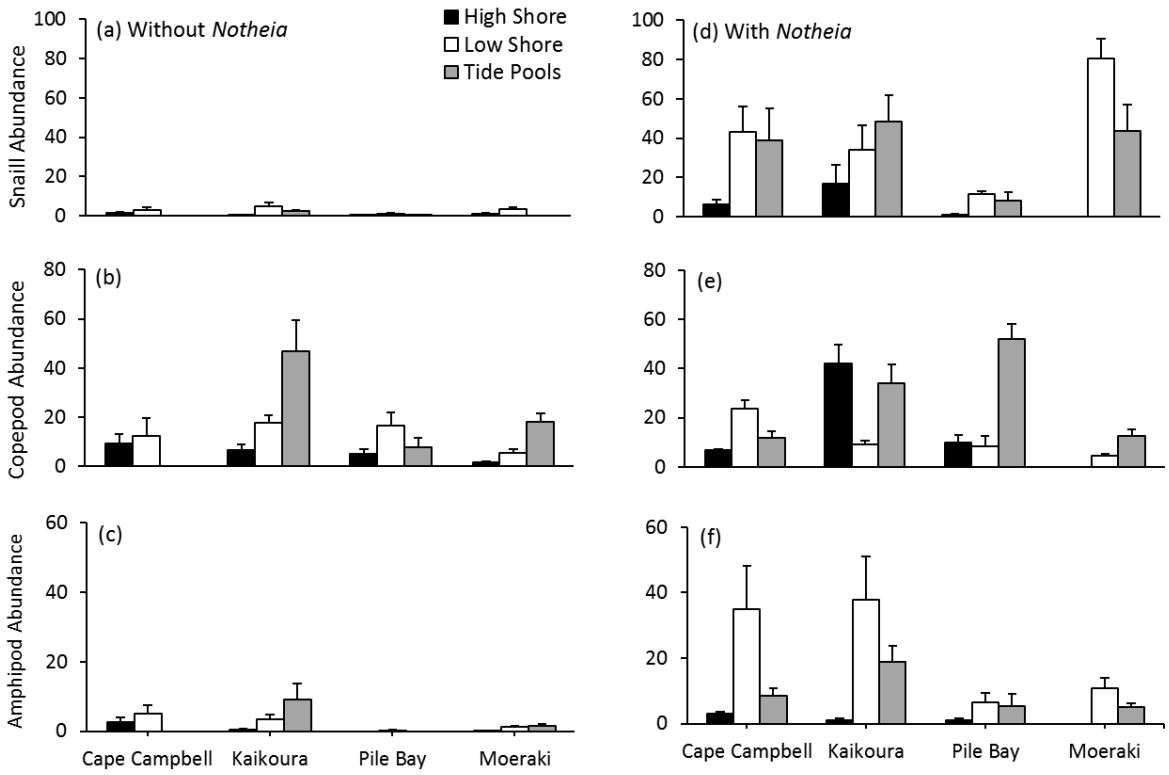

Figure 4.5. Total snail (a, b), copepod (c, d), and amphipod (e, f) abundance across four seasons at three shore elevations. Graphs on left are *Hormosira* samples without *Notheia* (a, b, c), graphs on right are with *Hormosira* samples with *Notheia* (d, e, f). *Notheia* was not present in the high shore at Moeraki, which is why there are no values. Error bars are standard error.

Table 4.2. The relationship between *Notheia* dry weight and invertebrate abundance and richness. See Thomsen et al. (2016) for comparisons between *Hormosira* and *Notheia*. All analyses were done using quasi-Poisson regression.

Region	df	Std. Error Abundance	Std. Error Richness	P-value Abundance	P-value Richness
Cape Campbell	16	0.073	0.062	<0.001	0.007
Kaikoura	233	0.023	0.013	<0.001	<0.001
Pile Bay	14	0.641	0.249	0.038	0.017
Moeraki	117	0.017	0.011	<0.001	<0.001

Temporal Survey:

A series of significant interaction effects highlighted that the abundance dynamics of these small invertebrates can be highly complex (Table 4.3). For example, while there was no significant effect of season on snail abundance, there was a significant elevation x season interaction. This could be because during fall, there was a steep drop in numbers in the high shore habitat compared to spring and summer. These values then remained low during winter. Another example can be seen in copepod abundances, where there is a significant epiphyte level x season and an epiphyte level x elevation interaction. The epiphyte level x season interaction can be seen in Figure 4.7e where even when the epiphyte is present, values are still considerably low in winter. The epiphyte level x elevation interaction is because copepod abundances were high in the tide pool habitat, even when there was no epiphyte present.

In samples where the epiphyte was present, all invertebrate richness and abundance tests were significant. Significant results of elevation were also calculated for each test (Table 4.3). A *post hoc* test (LSD) highlighted that these results were similar to the spatial survey in that all invertebrate abundance values were lowest in the high shore habitat and highest in the tide pools. Total invertebrate abundance values were highest in fall and summer, and lowest in spring and winter, and this was consistent across all diversity metrics tested, apart from snails (Figure 4.7).

Table 4.3. ANOVA results of *Notheia* epiphyte level (±), tidal elevation, and season on invertebrate diversity in Kaikoura. All invertebrate abundance values are standardised by host and epiphyte dry weight. Significant factors ($p<0.05$) are shown in bold. Levene's test for variance homogeneity are shown in brackets following each test factor.

Test	Treatment	SS	Df	F-Value	P-Value
Richness	Epiphyte Level (Epi)	133.4	1	81.81	**<0.001**
	Elevation (Ele) [0.09]	33.5	2	10.275	**<0.001**
	Season (Sea) [0.701]	115.2	3	23.557	**<0.001**
	Epi x Ele	3.6	2	1.112	0.331
	Epi x Sea	2.1	3	0.439	0.725
	Ele x Sea	21.5	6	2.201	0.442
	Epi x Ele x Sea	0.9	6	0.087	0.998
	Error	345.6	212		

Abundance	Epi	128006	1	51.859	**<0.001**
	Ele [<0.001]	78982	2	15.999	**<0.001**
	Sea [0.09]	30044	3	4.057	**0.008**
	Epi x Ele	6074	2	1.230	0.294
	Epi x Sea	13316	3	1.798	0.149
	Ele x Sea	16790	6	1.134	0.344
	Epi x Ele x Sea	10170	6	0.687	0.661
	Error	523286	212		
Snail Abundance	Epi	37455	1	41.505	**<0.001**
	Ele [<0.001]	14873	2	8.241	**<0.001**
	Sea [0.25]	5362	3	1.981	0.118
	Epi x Ele	8721	2	4.832	**0.009**
	Epi x Sea	3924	3	1.449	0.229
	Ele x Sea	12650	6	2.336	**0.033**
	Epi x Ele x Sea	3704	6	0.684	0.663
	Error	191317	212		
Copepod Abundance	Epi	4105	1	8.366	**0.004**
	Ele [0.044]	11345	2	11.561	**<0.001**
	Sea [0.74]	6186	3	4.203	**0.006**
	Epi x Ele	3593	2	3.662	**0.027**
	Epi x Sea	1577	3	1.072	**0.036**
	Ele x Sea	4478	6	1.521	0.173
	Epi x Ele x Sea	3105	6	1.055	0.391
	Error	104017	212		
Amphipod Abundance	Epi	7897	1	33.144	**<0.001**
	Ele [<0.001]	3084	2	6.471	**0.002**
	Sea [0.024]	3369	3	4.714	**0.003**
	Epi x Ele	1158	2	2.430	0.09
	Epi x Sea	2053	3	2.872	**0.037**
	Ele x Sea	3818	6	2.671	**0.016**
	Epi x Ele x Sea	2226	6	1.557	0.161
	Error	50513	212		

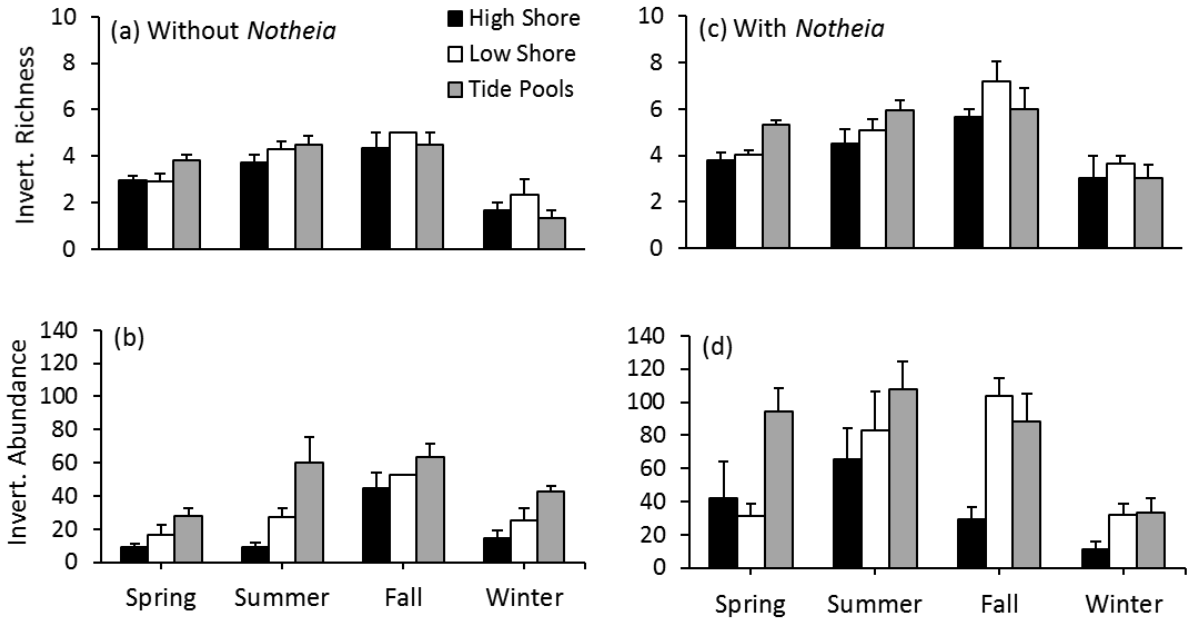

Figure 4.6. Invertebrate richness and abundance across four seasons, and three intertidal habitats at Wairepo Reef Kaikoura. Graphs on left are without *Notheia*, graphs on right are with *Notheia*. Invertebrate abundance values are standardised by host and epiphyte dry weight. Error bars are standard error.

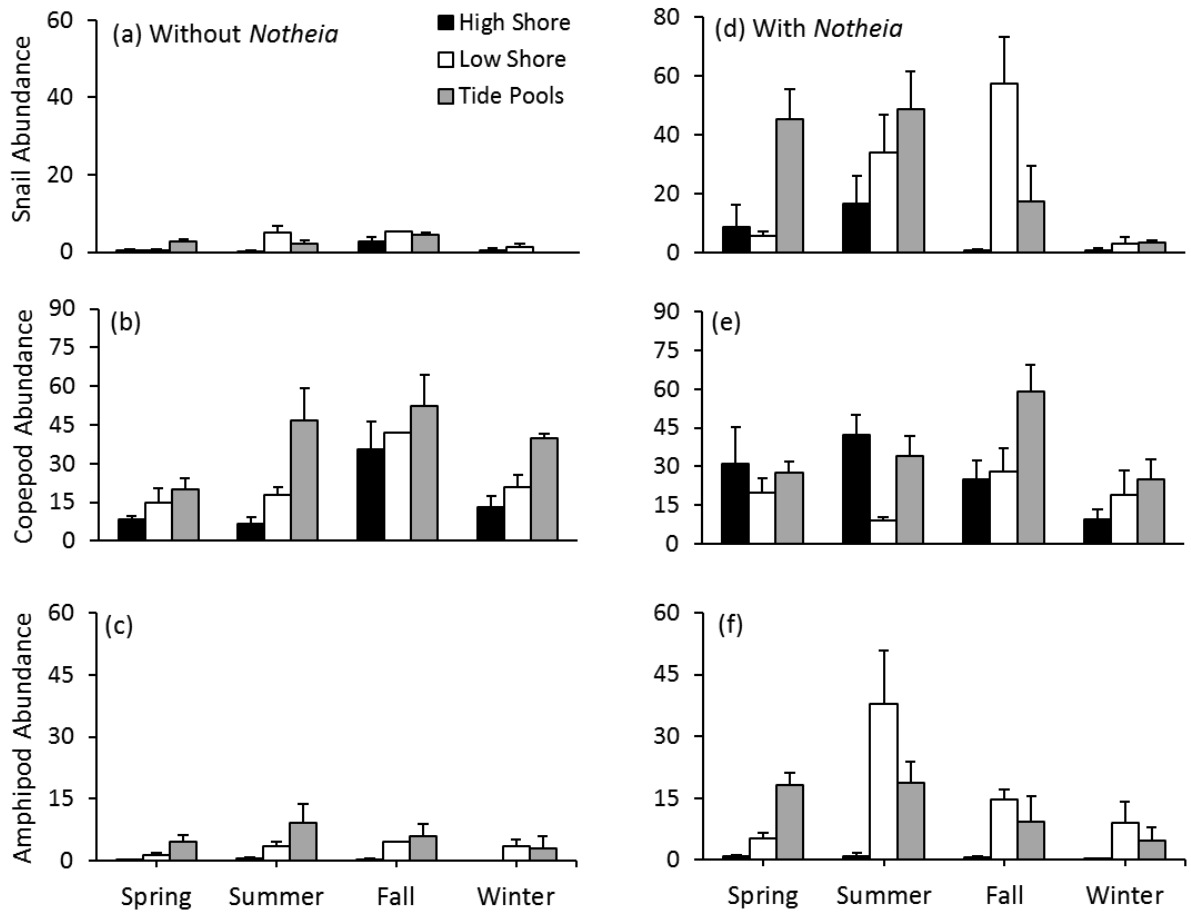

Figure 4.7. Snail abundance, copepod abundance, and amphipod abundance across four seasons, and three intertidal habitats at Wairepo Reef, Kaikoura. Graphs on the left are without *Notheia*, graphs on right are with the *Notheia*. All values are standardised by host and epiphyte dry weight. Error bars are standard error.

4.4.2. Mimic and density experiment

There were a series of interaction effects in this data set, such as a complex three-way epiphyte level x site x epiphyte level for total invertebrate abundance, gastropod, and amphipod abundance. This simply implies that ecological relationships are highly variable across factors such as tidal elevation, season and region, and that not all species will react the same to each factor. Significant differences between Wairepo and South Bay came up in all tests, which resulted in all interaction effects with 'Region' being significant (Table 4.4). There were much stronger differences between treatments in Wairepo than in South Bay.

There was a significant effect of epiphyte biomass (level) on all tests apart from invertebrate richness and copepod abundance, with this factor having the highest SS values of all (Table 4.4). Copepod abundances were high in the control treatment *Hormosira* (Figure 4.9). Epiphyte type had a significant effect on amphipod abundance only, and using a *post hoc* (LSD) it was highlighted that the artificial epiphyte facilitated higher amphipod abundances than the living epiphyte (Figure 4.9).

Table 4.4. ANOVA results of *Notheia* epiphyte level (low/high density), epiphyte type (live/artificial), and site (Wairepo/South Bay) on invertebrate diversity in Kaikoura. Significant factors ($p<0.05$) are shown in bold and near-significant effects ($p<0.10$) in parentheses. Levene's test for variance homogeneity are shown in brackets following each test factor.

Test	Treatment	SS	Df	F-Value	P-Value
Richness	Epiphyte Level [0.52]	12.99	2	3.183	(0.06)
	Epiphyte Type [0.027]	0.62	1	0.302	0.588
	Site [0.991]	21.3	1	10.441	**0.004**
	Epi. level x Epi. type	0.64	1	0.315	0.58
	Epi. Level x Site	6.31	2	1.548	0.234
	Epi. type x Site	5.5	1	2.698	0.114
	Epi. Level x Epi. Type x Site	0.27	1	0.132	0.72
	Error	46.92	23		
Total Abundance	Epiphyte Level [0.249]	86006	2	29.287	**<0.001**
	Epiphyte Type [0.224]	1571	1	1.07	0.312
	Site [0.036]	55306	1	37.666	**<0.001**
	Epi. level x Epi. type	4755	1	3.238	(0.085)
	Epi. Level x Site	12985	2	4.422	**0.024**
	Epi. type x Site	10815	1	7.366	**0.013**
	Epi. Level x Epi. Type x Site	23743	1	16.17	**<0.001**
	Error	33771	23		
Gastropod Abundance	Epiphyte Level [0.233]	19171	2	10.277	**<0.001**
	Epiphyte Type [0.352]	1	1	0.001	0.973
	Site [0.122]	8944	1	9.589	**0.005**

	Epi. level x Epi. type	812	1	0.870	0.361
	Epi. Level x Site	3608	2	1.934	0.167
	Epi. type x Site	4305	1	4.615	**0.042**
	Epi. Level x Epi. Type x Site	6274	1	6.727	**0.016**
	Error	21453	23		
Copepod Abundance	Epiphyte Level [0.84]	459.5	2	1.827	0.184
	Epiphyte Type [0.891]	95.9	1	0.762	0.392
	Site [0.005]	1537.9	1	12.228	**0.002**
	Epi. level x Epi. type	18	1	0.143	0.709
	Epi. Level x Site	96	2	0.382	0.687
	Epi. type x Site	108	1	0.859	0.364
	Epi. Level x Epi. Type x Site	367	1	2.918	0.101
	Error	2892.5	23		
Amphipod Abundance	Epiphyte Level [0.006]	8462	2	29.776	**<0.001**
	Epiphyte Type [0.05]	1801	1	12.675	**0.002**
	Site [0.487]	1812	1	12.754	**0.002**
	Epi. level x Epi. type	1023	1	7.199	**0.013**
	Epi. Level x Site	662	2	2.328	0.12
	Epi. type x Site	313	1	2.2	0.15
	Epi. Level x Epi. Type x Site	1108	1	7.8	**0.01**
	Error	3268	23		

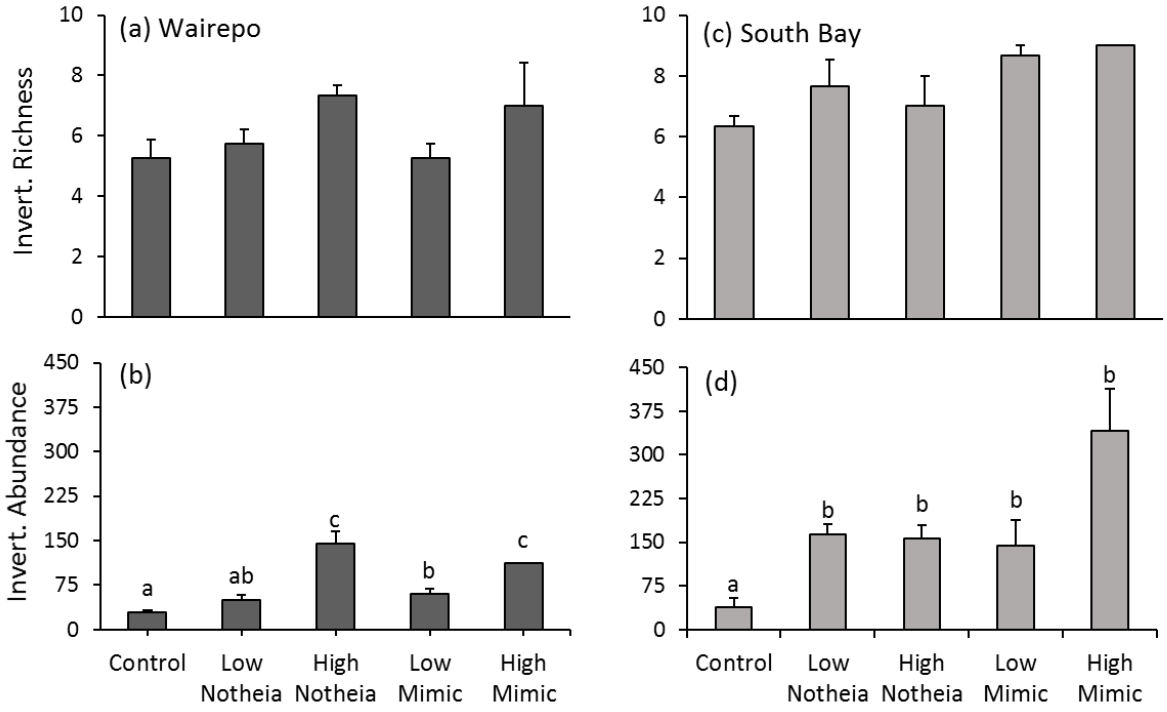

Figure 4.8. The effect of experimental treatments on invertebrate richness (a,c), and invertebrate abundance (b,d). Wairepo N= 4 for each treatment, apart from "High *Notheia*" which is 3. South Bay N= 3 for each treatment, apart from "High Mimic" which is 2. Error bars are standard error.

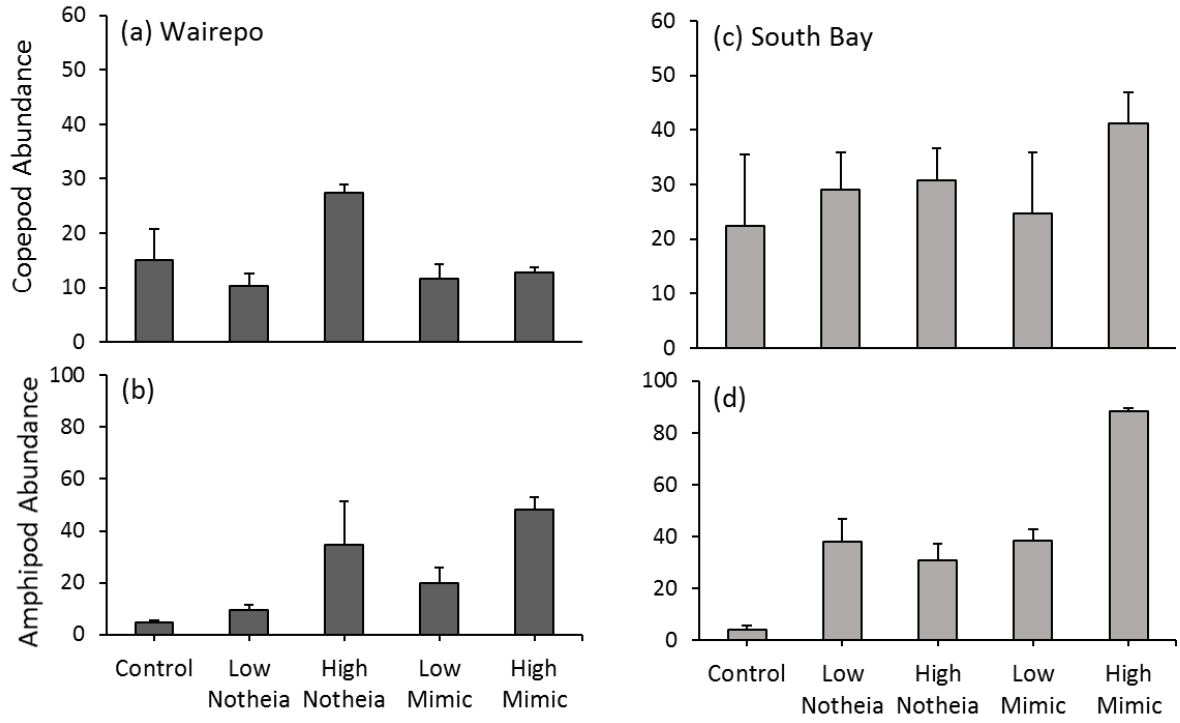

Figure 4.9. The effect of experimental treatments on total copepod abundance (a,c), and total amphipod abundance (b,d). All values are standardised by host and epiphyte dry weight. Wairepo n= 4 for each treatment, apart from "High *Notheia*" which is 3. South Bay n= 3 for each treatment, apart from "High Mimic" which is 2. Error bars are standard error.

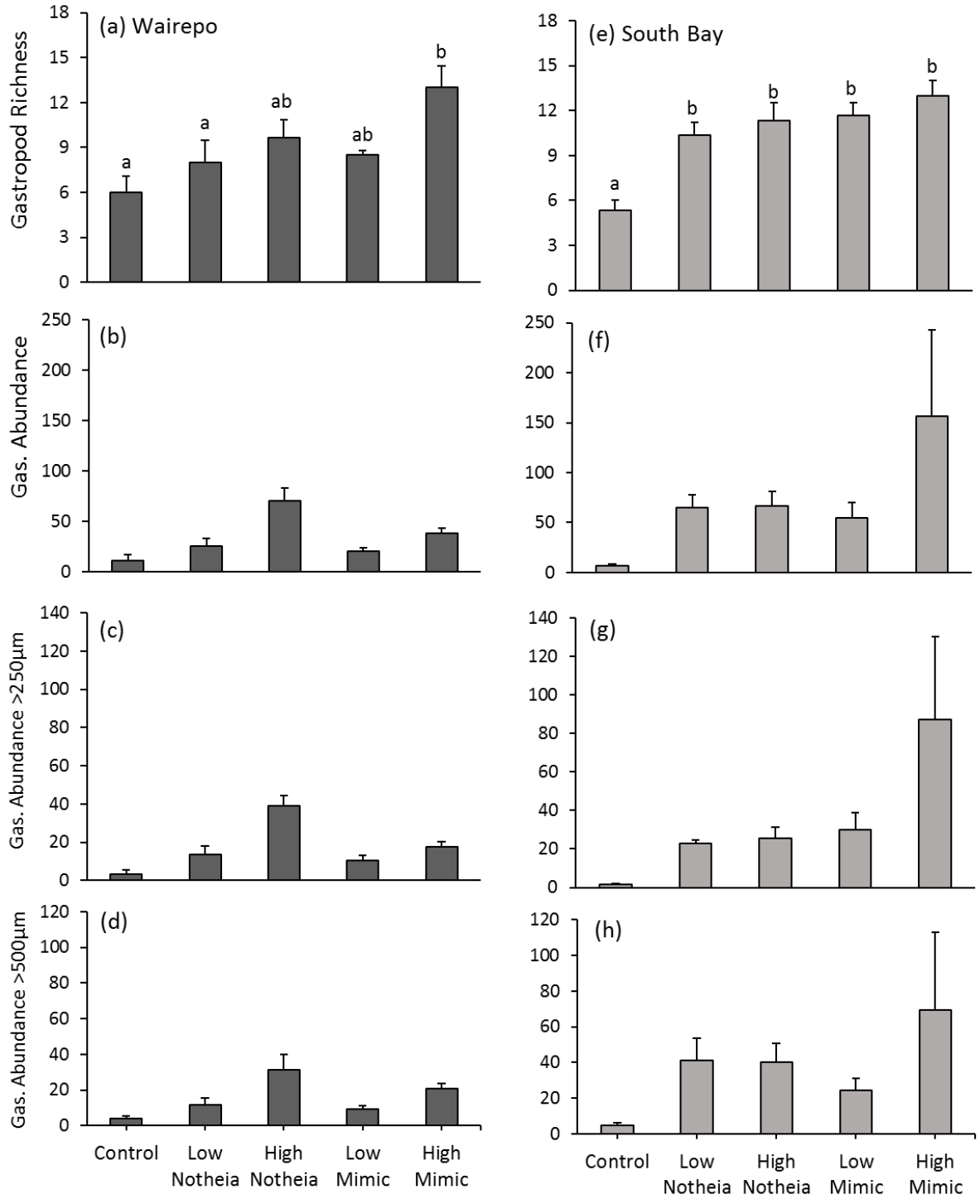

Figure 4.10. The effect of experimental treatments on gastropod richness (a,e), total gastropod abundance (b,f), total gastropod abundance 250-500 µm (c,g), and total gastropod abundance >500 µm (d,h). All gastropod abundance values are standardised by host and epiphyte dry weight. Wairepo n= 4 for each treatment, apart from "High *Notheia*" which is 3. South Bay n= 3 for each treatment, apart from "High Mimic" which is 2. Error bars are standard error.

4.4.3. Snail grazing experiment

There was a significant effect of snail grazing on *Hormosira* and *Notheia* in both the mono and mixed experiment (Table 4.5). There was a near-significant effect of species, suggesting that with higher sample sizes, grazing might affect one species of seaweed more strongly than the other. The ANOVA was performed on change in weight (g), whereas Figure 4.11 is shown as percent change in biomass.

Table 4.5. ANOVA testing gastropod grazing on *Hormosira* and *Notheia* in a mono and mixed experiment. Significant factors ($p<0.05$) are shown in bold, near-significant factors are shown in parentheses. Levene's test for variance homogeneity are shown in brackets following each test factor.

Test	Treatment	SS	Df	F-Value	P-Value
Change in biomass	Species (Spp.) [<0.001]	43.83	1	4.003	(0.057)
	Grazing Effect [0.383]	72.08	1	6.584	**0.017**
	Experiment (Exp.) [0.759]	0.03	1	0.003	0.958
	Spp. x Grazing	46.28	1	4.227	(0.051)
	Spp. x Exp.	0.16	1	0.015	0.905
	Grazing x Exp.	0.91	1	0.083	0.776
	Spp. x Grazing x Exp.	1.14	1	0.104	0.75
	Error	262.75	24		

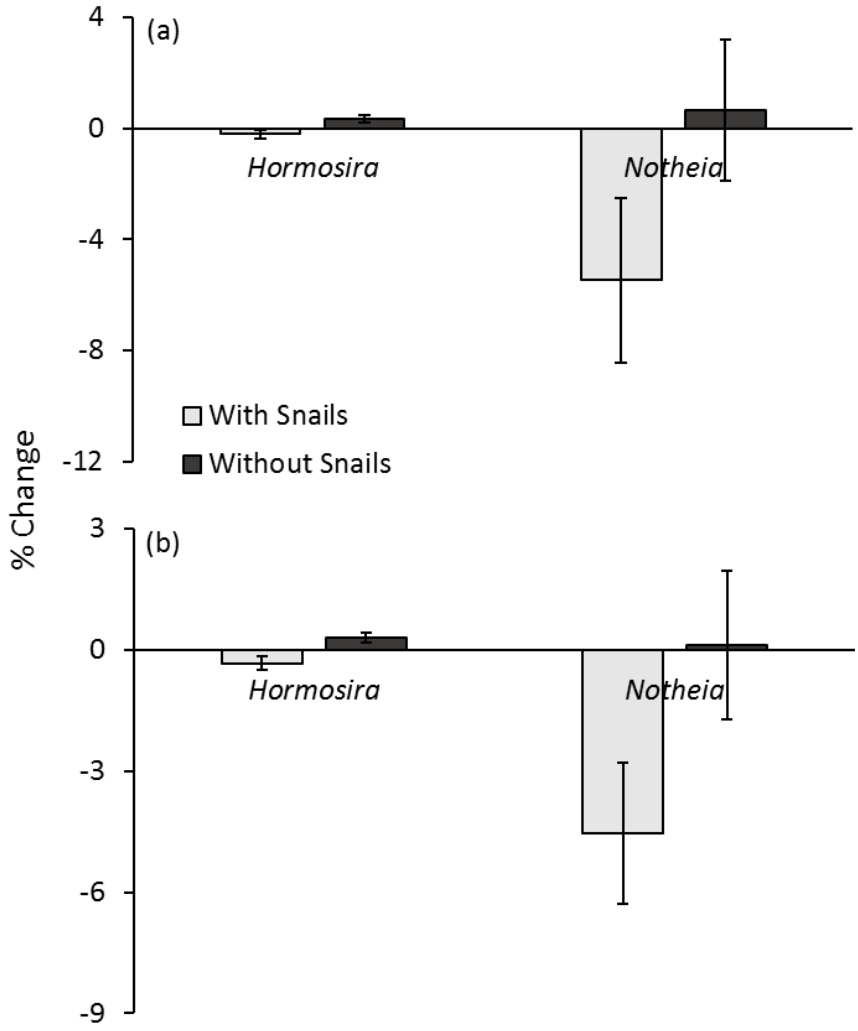

Figure 4.11. The effect of snail grazing on either *Hormosira* or *Notheia* % change in biomass when they were presented independently (a), and when they were presented together (b). Error bars are standard error; n= 4.

4.5. Summary

Most studies looking at facilitative effects of epiphytes have focused on epiphytes with low host specificities (Hall and Bell 1988, Edgar and Robertson 1992, Martin-Smith 1993), or sampled from few environments, places, or temporal events (Hallam et al. 1980, Thomsen et al. 2016). Furthermore, little is known about processes that underpin and maintain or inhibit the facilitative effects that the epiphyte may be providing.

Thomsen et al. (2016) showed that a facilitative relationship between *Hormosira* and *Notheia* had positive effects on invertebrate communities in the intertidal zone. My study incorporated

a long-term sampling protocol, and added more regions across the South Island. The findings were consistent with those made by Thomsen et al. (2016), and the bigger data set gave new and interesting results. Across all four seasons and at four intertidal regions there were strong positive effects of *Notheia* biomass on invertebrate biodiversity, implying the ecological importance of *Notheia* as a provider of habitat. There were a series of differences among invertebrate taxa, for example amphipod abundance was highly linked to high biomass of the epiphyte, whereas copepod abundance was high even in *Hormosira*-only samples. Snail abundance was greatest in high *Notheia* samples, but this was only true in the warmer months. In general, invertebrate taxa were most abundant when the epiphyte was present and in high abundance.

In an attempt to further understand the effect of epiphyte density on invertebrate diversity I used a recolonization-experiment at Wairepo Reef and South Bay in Kaikoura looking at differences between artificial and living epiphytes. I found strong effects of the high density epiphyte treatments (similar to the results from Hall and Bell (1988)) on invertebrate diversity in all Wairepo Reef, and most South Bay samples. There was a significant effect of epiphyte biomass (level) on all tests apart from invertebrate richness and copepod abundance. *Notheia* is much more branched and structurally complex than *Hormosira* which is why we see these positive relationships. Copepod abundances were high in the control treatment, which suggests that *Hormosira* is a suitable habitat for these taxa. For amphipods, the artificial epiphyte at high density hosted more individuals than the living epiphyte at high density.

Finally, I questioned whether the invertebrate grazing communities have a significant effect on *Notheia* biomass. I ran an experiment where I measured *Notheia* and *Hormosira* biomass before and after snail grazing pressure. I found negative effects of grazing on both *Hormosira* and *Notheia*, with stronger grazing on *Notheia*. However, grazing rates were low overall, indicating that under natural field conditions, other stressors such as light limitation, nutrient stress, desiccation and storms, are likely to swamp grazing effects. Supporting the colonization experiment, the grazing experiment suggests that *Notheia* primarily provides a better habitat for invertebrates.

In conclusion, my results support a growing number of habitat cascade studies from different ecosystems, and suggest that these types of processes are common in marine benthic systems where epibiosis is a common occurrence. Because there is relatively little evidence of

substantial feeding by invertebrates on *Notheia*, it is likely that the extra habitat provided by the epiphyte is the primary resource facilitating invertebrates.

Chapter Five

General Discussion

Very few studies have described the unique relationship between the obligate epiphytic brown alga *Notheia anomala* and its host *Hormosira banksii* even though this interaction is common on large biogeographical scales (Chapter Three, Figure 3.5). My project tested how a variety of phenological, physiological, and ecological factors affect the relationship between host and epiphyte, and how their co-occurrences further affect higher trophic levels. In this chapter I will discuss the major findings from my studies, starting with *Notheia* reproduction, then going on to the association between *Notheia* and *Hormosira*, and finishing with the way in which these seaweeds affect higher trophic levels such as their invertebrate inhabitants.

5.1. The reproductive dynamics of Notheia anomala

Notheia is a seaweed found growing abundantly on *Hormosira* plants in areas with low desiccation stress such as the low shore and tide pools (Hallam et al. 1980). In Kaikoura its abundance drops slightly (but not significantly) in winter (Chapter Three), but it is still relatively abundant year-round. It is therefore surprising that I found distinctive patterns in reproduction during the sampling period of October 2015 to August 2016. Among the high shore, low shore, and tide pool habitats, there was a distinct quiescent period from July to October where most individuals had sterile conceptacles (Stage I). April demonstrated peak reproduction among habitats (Stage VI), as individuals sampled during this month had conceptacles filled >90% with both male and female gametes. Stage II and III individuals occurred throughout the rest of the year, with stage IV and V generally appearing from December to June, but unlike Stage I and VI, there were large differences in the proportions of these intermediate stages among habitats.

These reproductive patterns are likely to be linked to the coastal sea water temperature in the Kaikoura region (Chapter Two, Figure 2.5). Many species worldwide demonstrate reproductive patterns that are closely tied with temperature (Bale et al. 2002, Brown et al. 2004, Nutsford 2010). Begum and Taylor (1991) showed a clear correlation between liberation and temperature for *Hormosira*, as oogonia are released when sea water temperature decreases after summer. Highest levels of fertilisation were recorded from July to October relating to winter and early spring, and highest egg release occurred in seawater temperatures of 13.5-16 °C (Begum and Taylor 1991). In April 2016 (Autumn) when *Notheia* conceptacles were most developed, the coastal seawater temperatures were 14.9-15.5 °C.

While there were distinctive patterns in *Notheia* reproduction across seasons (from October 2015 to August 2016), it was initially predicted that they would be different among intertidal habitats (i.e. the high shore, low shore, and tide pools). This prediction was consistent with what I found. During peak reproduction in April, the low shore populations had a lower proportion of individuals at stage VI (17% of individuals) than the high shore (60%) and tide pool (67%) populations. Although desiccation stress is higher in the high shore environment, it could be that strong wave forces down in the low shore are knocking off the larger, more reproductive individuals. There was a peak of Stage V individuals in the high shore (40% of individuals) and tide pools (100% of individuals) in December, but this peak was not observed in the low shore. Again, while it was initially expected that desiccation would be a factor limiting development in the high shore only, there may be other factors such as wave energy, or competition with larger more dominant species, that is affecting the low shore *Notheia* population differently to the high shore and tide pools.

Stage III, where conceptacles are half filled with female gametes only, was never present in the tide pool population, with one peak in the high shore from November – January, and two peaks in the low shore in December and June. The tide pool populations experience the lowest level of desiccation stress, and therefore may be able to allocate more energy into reproduction and growth. This may allow individuals to develop faster than the individuals in the high and low shore.

There are very few studies that have tested asynchronous reproduction patterns in the same species (including algae) in different intertidal habitats. Alternatively, a study by Kelly and Metaxas (2007) assessing reproduction in the deep sea hydrothermal vent limpet *Lepetodrilus fucensis* found that fewer individuals from senescent areas displayed gametogenic maturity than individuals from vigorous, diffuse, and peripheral areas and areas among tubeworm bushes. The researchers hypothesised that the multiple feeding strategies of *L. fucensis* could allow for a constant supply of energy to be allocated to reproduction in all habitats except senescent vents. Again, it is likely that *Notheia* living in tide pools experiences less environmental stress, and is therefore able to allocate a lot more energy into reaching reproductive maturity faster and for longer.

While thousands of years of co-evolution have left *Notheia* completely reliant on *Hormosira* for growth and survival (Silberfeld et al. 2010), it appears to have a slightly different period of reproduction to its host. In nature there is always a trade-off between allocating energy to

growth and survival, and allocating it to reproduction (Stearns 1989). *Notheia* may be allocating more energy into growth in the warmer months when daylight hours are long and solar radiation high (Chapter Three), and then as temperatures begin to cool, they change their energy allocation into reproduction. During these cooler months there is a higher likelihood of storm events occurring, resulting in a significant loss of biomass for most intertidal seaweeds (Gremare et al. 2003, Scott et al. 2016). It could be that over evolutionary time scales, *Notheia* has adapted to these events so that reproduction occurs during winter when storms are more intensive and biomass may be lost during those events anyway. It is likely that the larger *Notheia* individuals have a higher reproductive output because they have more tissue for conceptacles to develop in. However, large individuals do not dominate the population. Therefore, it is the medium sized individuals that are providing a higher output as a whole because there more of them. Medium sized individuals also have a lower risk of dislodging from their host due to lower drag, and are therefore less likely to lose significant biomass during a storm event.

A limitation in this research lies in the sampling procedure for this study. In the high shore habitat at Wairepo Reef, there are areas which can be lower in elevation than predicted due to the variable sloping nature of the platform. There are also many small cracks and crevices that may be holding water for longer than other areas in the high shore, therefore *Notheia* individuals living near these areas may be experiencing lower levels of desiccation stress and are therefore not truly representative.

To improve this study in the future reproduction should instead be quantified with numerical data, for example, using the percent of mature female and male gametes (Kelly and Metaxas 2007) or percent cover of reproductive structures within an individual receptacle, although the spherical shape of the conceptacle, and therefore the loss of data in a 10 µm section should be taken into account. More resources should be allocated into collecting and processing more samples. A minimum of 10 samples per habitat per month would be more sufficient to reduce variation and allow clearer conclusions. Only processing every alternate month in this study allows for gaps in the story. Finally, it would be interesting to add another site or region to the analysis. For example, also collecting individuals from South Bay, or further down south in Moeraki, would add a great deal of information as to whether reproduction is being strongly influenced by temperature or wave energy.

5.2. The distribution and interactions between the obligate epiphyte Notheia anomala and its canopy forming host Hormosira banksii

In epiphytism, ecological relationships between host and epiphyte can be highly variable, and the nature of the relationship can change across ecological transition zones such as tidal elevation, seasonal cycle, and latitude (Callaway et al. 2002, Thornber et al. 2016). Understanding the ways in which these factors affect epiphytic relationships is fundamental to understanding the life strategies of epiphytic organisms as a whole. The continental-scale distribution of *Hormosira banksii* and *Notheia anomala* are documented in a variety of different sources (Harvey 1860, Osborn 1948, Guiry and Guiry 2016), however these references only explain the extent of their distributions, and not the nature of *Notheia* absences where *Hormosira* is present. By extracting information from the New Zealand and Australian herbarium, I found that while *Notheia* distribution is intricately linked to its obligate host *Hormosira* across Australasia, but there were gaps it its occurrence which may, in part, be explained by high temperature (and desiccation) stress at low latitudes.

Distribution and abundance on vertical, regional, and temporal scales

The distinct vertical patterns that seaweeds exhibit in the intertidal zone have interested ecologists and phycologists for over a century (Cranwele and Moore 1808, Colman 1933, Zaneveld 1937, Broekhuysen 1940). When quantifying the vertical distribution patterns of *Hormosira* and *Notheia* at Moeraki and Kaikoura, I found contrasting distributions among the two species: *Hormosira* was least abundant and *Notheia* was most abundant in tide pools (Thomsen et al. 2016). I also found that there were differences in canopy covers between the regions. In Kaikoura, *Hormosira* was more abundant in the low shore, and *Notheia* was less abundant in the tide pools. It has been suggested that *Hormosira* is one of the most desiccation-resistant canopy formers in temperate Australasia, allowing it to dominate higher reaches of the intertidal zone (Underwood 1998, Bellgrove et al. 2010). But at lower tidal elevations, larger and faster growing canopy formers such as *Cystophora* spp. typically outcompete *Hormosira* (Schiel 2006). In comparison to *Hormosira*, I hypothesised that *Notheia* is more susceptible to desiccation stress, which is why it is limited to growing on *Hormosira* fronds in wetter environments such as tide pools and the low shore (Hallam et al. 1980). When analysing temporal changes in abundances, neither *Notheia* nor *Hormosira* exhibited significant seasonal

variation over one year, but contrasting elevation patterns were again observed. *Hormosira* is a perennial species, that survives for longer than seasonally occurring 'annual' species (Schiel 2011), and it is therefore possible that due to thousands of years of co-evolution, *Notheia* displays a similar life strategy to its obligate host.

Distribution and abundance on host plant scales

Small-scale attachment of *Notheia* varied among elevations. The greatest number of *Notheia* clumps in the high shore was attached to the low region of *Hormosira* hosts. By contrast, most *Notheia* clumps on the low shore and in tide pools were attached to the mid and high regions of the host. In the high shore, *Notheia* is likely to survive better low in the *Hormosira* canopy where moisture levels likely are greatest. Alternatively, in the low shore and tide pools, desiccation stress is lower but low-light stress higher, and it is therefore more advantageous to be attached higher up in the canopy (Davison and Pearson 1996).

The most common site for *Notheia* attachment were the nodes on which *Notheia* attached around the outer edge of the ostiole, rather than inside the reproductive conceptacle itself as previously stated (Harvey 1860). Fundamental reliance on reproductive structures for survival is therefore not expected. Furthermore, I found that *Notheia* does not associate with only one sex of *Hormosira*, but were found attached to both male and female host plants in equal proportions (Hallam et al. 1980, Ducker and Knox 1984).

The tissue connection between *Hormosira* and *Notheia* is very close, but there is no protoplasmic association (Hallam et al. 1980). Sexually mature *Hormosira* plants had many more attached *Notheia* plants compared to immature *Hormosira* (Harvey 1860, Hallam et al. 1980), perhaps because as *Hormosira* gets older there is a degenerative effect on the protective functions that are put in place to prevent epiphytic attachment (Hall and Bell 1988, Durante and Chia 1991, Jennings and Steinberg 1997). Furthermore, Hall and Bell (1988) found that old seagrass blades have larger numbers of epiphytic algae, simply because there is more time for colonization, compared to younger leaves. In intermediate and old-aged *Hormosira* individuals *Notheia* was more frequently attached to the nodes of the *Hormosira* as opposed to the internodes (and the attachment is not always directly to an ostiole, personal observation). This is probably explained simply because the nodes have much larger surface area than the

internodes. However, *Notheia* was more frequently attached to internodes of young *Hormosira* individuals, perhaps suggesting that younger individuals have more defences to prevent epibiosis near the reproductive structures (Durante and Chia 1991, Jennings and Steinberg 1997).

Survival and growth

A tagging experiment performed at Wairepo Reef tested growth and longevity of *Notheia*. There was a high loss of samples over a five-month period in both the low shore and tide pool habitats, with >75% of all tagged plants lost by the fifth month. Loss of samples was often due to the dislodgement of *Hormosira* rather than *Notheia*. Because tagging was done in winter, it is likely that the more frequent winter storms was a major contributor to the high recorded dislodgement of *Hormosira*. In addition, *Hormosira* fronds with large and dense *Notheia* have high drag and therefore an increased risk of dislodgement (although, I found that control fronds where tags were attached to un-epiphytised *Hormosira* had equally high mortality and dislodgement rates). However, even though most samples were lost over five months, my seasonal distribution data (Chapter Three) showed that its abundance is stable throughout the year, suggesting that the population have mechanisms that allow fast recovery after dislodgement.

The tagging experiment also showed that *Notheia* growth was not density-dependent. For example, larger clump *Notheia* treatments did not have a greater growth rate than smaller clumps. By contrast, most other seaweed species, such as the green alga *Ulva* sp., have density dependent growth (Viaroli et al. 1996). While not significant in the statistical analysis, I frequently observed that *Notheia* clumps of more than 5 cm length, when trimmed back to a few mm in length, often died back from its host. This die-back could be due to stress associated with the trimming, because it may decrease resistance to desiccation stress, or because these small trimmed fronds had insufficient tissues for production. The tagging experiment also suggested that large clumps in tide pools maintained biomass better than large clumps from the low shore. Again, the dataset had a large amount of variation, and these differences were not statistically significant.

One key shortcoming was that this experiment was performed only in the winter season. If the experiment was performed in summer the results would likely have been different, with less storm associated mortality and higher growth associated with increased light and higher temperatures. In September I put extra tags out to address the shortcoming of the winter experiment, but a catastrophic 7.8 magnitude earthquake in October uplifted all reefs along the Kaikoura coastline and destroyed virtually all *Hormosira* and *Notheia* populations overnight (personal observation). Future follow-up studies should include higher sample sizes, a longer sampling period and inclusion of all seasons. My experiment used a simple width x height measurement of individual tagged *Notheia* clumps (assuming a rectangular shape), to record growth. Measuring growth rate can be done in different ways, for example Pedersen et al. (2005) looks at percent tissue growth using surface area (as in my translocation experiment), whereas Viaroli et al. (1996) compare wet weights. These methods, however, are hard to do on small epiphytes and virtually impossible to do on epiphytes attached to host plants. Therefore, photographs to measure surface area are perhaps more accurate than my simple estimations.

Stress assays (translocation experiments)

Low *Notheia* biomass in the upper *Hormosira* zone is most likely due desiccation stress, the dominant factor shaping where marine organisms occur in the intertidal zone (Davison and Pearson 1996, Schiel 2006). A translocation experiment is a simple way to test if adult *Notheia* fronds can survive in the higher zone (Lipkin et al. 1993, Davison and Pearson 1996, Blanchette 1997), but no studies have transplanted *Notheia* to test if adult fronds can survive or grow in different intertidal habitats. The idea of transplanting seaweed however, is not novel and studies have used translocations to assess the effects of wave energy (Blanchette 1997), grazing pressure (Poore et al. 2009), and desiccation (Chapman and Johnson 1990, Lipkin et al. 1993) on algal distributions. For example, Blanchette (1997) found that mean size of wave-exposed *Fucus gardneri* (rockweed) increased significantly when it was transplanted to more protected sites. It is important to realize that, although transplant experiments may provide support for the hypothesis that physical factors are responsible for the observed upper distributional limits of certain species, they do not test alternative hypotheses such as the failure to recruit higher on the shore (Davison and Pearson 1996).

My results highlighted some of the physical aspects shaping *Notheia* distribution in the intertidal zone. First, I found that low shore *Notheia* populations translocated to the high shore do not survive. This result aligns with the natural distribution of *Notheia* that are much reduced in the high shore and indicates that desiccation is likely a key limiting factor.

Fronds transplanted to the low shore showed that *Notheia* growth was possible also when detached from its obligate host both artificially attached to another seaweed (*Hormosira* or *Cystophora*) or to bare rock (in a mesh bag). This suggests that once *Notheia* has settled on *Hormosira* and grown to a minimal size where it can photosynthesise on its own, it is capable of surviving without the host. Once it has become detached, it does not show the capability to reattach (but survival in a natural setting may still be possible if entangled on-shore). Gibson and Clayton (1987) demonstrated that male and female gametes of *Notheia* fuse only when the female gamete has become attached to the surface of its host (*Hormosira* or *Notheia*), and a chemosensory detection mechanism is suggested for the selection of host substrate. Being attached to bare substrate resulted in high *Notheia* mortality, indicating that canopy cover from a host plant has a strong influence on survival. In the low shore and tide pools I regularly saw *Notheia* attached to *Hormosira* beads higher in the canopy (see results section, Chapter Three), but the tide pools translocation experiment demonstrated that growth rates did not differ between low and high regions in the canopy. However, this experiment was only performed in the tide pools, and it is possible that there are stronger canopy effects in the low shore. Alternatively, the experiment was simply too short to detect significant differences in growth rates.

In general, the experiments conducted in this study showed high variation in both the survival of tagged plants and growth rates. This was in part because replication levels were relatively low (and reduced even further because of high loss of samples during the experiments). In future studies, sample sizes should be increased, and the study repeated in multiple seasons, as growth rates have been shown to vary dramatically between hot summers with high light levels and cold winters with low light (Todd and Lewis 1984, Potin et al. 1990).

In conclusion, while *Notheia* distribution is intricately linked to its host *Hormosira*, there are significant factors altering where it's found in the intertidal zone. It is likely that *Notheia* is not as tolerant to desiccation as its host, restricting it to hosts inhabiting wetter environments such as the low shore and tide pools. In extreme environments such as the high shore, it tends to grow only as very small fragments, found attached to the base of its host (where most of the

moisture is held during emersion periods). However, *Notheia* populations are resilient (present year round in most quadrats) despite very high turnover rates. Most growth is likely to be in summer when daylight hours are longest, light levels and temperature highest and storm events less frequent. Recognising the distribution and growth patterns of *Notheia*, from large scales right down to attachment point help us to understand the main mechanisms driving epiphytism, and how an epiphytes life strategy may change across spatial and temporal scales.

5.3. Notheia as a contributor to local-scale diversity

This research has consistently documented positive effects of the obligate epiphyte *Notheia anomala* on the diversity of small mobile invertebrates on intertidal rocky reefs from the South Island of New Zealand. There was strong evidence to support the hypothesis that facilitation would be strongest when *Notheia* biomass was greatest. Field experiments using live *Notheia* and an artificial mimic of its complexity demonstrated that the facilitative effect of *Notheia* is primarily due to the added structure and complexity as opposed to it being a food source. Additionally, I found that facilitation was strongly density-dependent with positive relationships between *Notheia* biomass and invertebrate diversity, a result also documented for epiphytes on seagrasses (Hall and Bell 1988) and seaweeds entangled around mangrove roots (Bishop et al. 2009, Bishop et al. 2012). Similar positive effects of epiphytes on invertebrates have also been documented in rocky intertidal systems (Pavia et al. 1999), and many other ecosystems including subtidal seaweed beds (Martin-Smith 1993), terrestrial forests (Watson 2002, Ellwood and Foster 2004), and seagrass beds (Hall and Bell 1988, Gartner et al. 2013).

Spatio-temporal distribution of mobile invertebrates

The strong facilitative effect of *Notheia* on invertebrate richness and abundance was consistent for all spatio-temporal surveys and manipulative experiments. Similar facilitative results were found for dominant lower-resolution taxonomic units including gastropods, copepods, and amphipods, although tests on specific taxonomic groups often displayed complex interactions between epiphyte presence, elevation, season, and region. For example, large numbers of snails and amphipods were only collected from samples containing very large biomass of *Notheia*. By contrast, copepods were more abundant on *Hormosira* fronds without epiphytes. It is likely

that different taxa use different structural parts of both *Hormosira* and *Notheia*, and copepods may be better adapted to feed on the mucilage or microalgae found on *Hormosira* than other species which instead require the structurally complex *Notheia* to escape predators (Turner 2004, Calbet et al. 2007). Several studies have demonstrated that high structural complexity of an alga may decrease the efficacy of a predator's capture of prey, in this case the prey being small invertebrates (Dionne and Folt 1991, Warfe and Barmuta 2004, Zamzow et al. 2010).

Invertebrate richness and abundance was often different among tidal elevations. Low shore and tide pool *Hormosira* and *Notheia* were typically inhabited by many more invertebrates than high shore populations (and values were standardised by host dry weights). There were also significant effects of season on invertebrate inhabitants, with nearly all diversity metrics being higher in the warmer seasons (i.e., summer and fall). This was especially true for snails, which were at very low numbers in winter, even when epiphyte levels were still relatively high.

Mimic and density experiment

The mimic experiment highlighted that seaweed biomass is important for invertebrate diversity, and that for almost all invertebrate taxa the physical structure of the epiphyte, rather than its biological properties were more important. These findings contrast those of Bologna and Heck (1999) who found that abundances of invertebrates were higher on mimic seagrasses with natural epiphytes, compared to mimic seagrasses with artificial epiphytes, implying that food subsidy was more important in this seagrass system.

There was a series of complex three-way interaction effects in the statistical analysis, which implies that ecological relationships are highly variable across tidal elevation, season and region, and that not all species reacted the same to each factor. There was significant spatial variability among sites and treatment effects were consistently stronger at Wairepo Reef than in South Bay. However, differences between Wairepo and South Bay should be treated with caution, because sample sizes were smaller at South Bay. It was interesting that amphipod abundances were higher in the artificial treatments than the living treatments. It suggests that substrate complexity is more important for amphipods than the biological properties that a living epiphyte provides, as high structural complexity of an alga can decrease the predator's

potential to detect prey, and increase the likelihood for prey escape (Sotka et al. 1999, Zamzow et al. 2010, Navarro-Barranco et al. 2013).

The period allowed for recolonization could potentially have implications for the final results. For example, Hall and Bell (1988) only needed three days to document colonisation numbers similar to surveyed plants. However, Martin-Smith (1993) used 100 days, and saw differences in community structures between two and four weeks of field incubation. However, a longer time period allows sessile organisms to attach to artificial surfaces, thereby making the mimics more like living substrates. Perhaps if my experiment was left out for longer than two weeks, the number and richness of inhabitants might have become more similar among treatments (Bologna and Heck 1999). My experiment was also only performed in one season (December 2015, Summer), and it is possible that results may vary across temporal gradients due to lower temperature and stronger storms in colder months. I lost many more samples from South Bay than Wairepo, probably because wave energy is stronger on the south side of the Kaikoura Peninsula. Analysis of gastropod responses (with higher taxonomic resolution) demonstrated relatively similar effects, as analysis carried out on course taxonomic units. More specifically, richness of gastropods was quantified using 'morpho-types' classified based on cone height, spiral patterns, and colour. When identifying these morpho-types, I cannot be sure whether a morpho-type in the 250 µm-500 µm samples simply was a juvenile of another morpho-type found in the >500 µm samples. This is a weakness of the study, but simple taxonomic keys do not exist to cover all the observed micro-gastropods, and my conclusions about strong density-dependent effects of *Notheia* is valid irrespective of the true taxonomic identity of these grazers.

Overall, increased complexity and density of epiphytes had strong positive effects on invertebrate diversity, and *Notheia* facilitates this by being more morphologically complex than its host *Hormosira*. More complex habitats typically increase survival and biodiversity in highly competitive environments such as shallow rocky intertidal systems (Russo 1987, Dionne and Folt 1991, Warfe and Barmuta 2004, Zamzow et al. 2010, Bishop et al. 2012).

Snail grazing experiment

Results from a laboratory gastropod grazing experiment suggested that gastropods can consume both *Notheia* and *Hormosira*, with slightly stronger grazing effects on *Notheia*. However, the net effect of grazing was low overall. This indicates that under natural field conditions, other stressors such as light limitation, nutrient stress, desiccation and storms, are likely to affect *Notheia* biomass far more than the small grazing effects from micro-gastropods. These results contradict the mimic experiment, where invertebrate inhabitants (except amphipods) used living and mimic *Notheia* similarly (suggesting that *Notheia* provides only habitat space). Previous studies on habitat cascades have consistently highlighted positive effects of habitat formation on inhabitants (Hall and Bell 1988, Edgar and Robertson 1992, Thomsen et al. 2010, Bishop et al. 2012), but my study is one of only a few that have documented negative feedbacks from the inhabitants to both the hosts and epiphytes demonstrating a series of positive and negative relationships within a habitat cascade (Figure 5.1).

There were several limitations to the grazing experiment. First, sample sizes were relatively small, resulting in relatively large variation in grazing effect. Furthermore, it is difficult to estimate precise wet weights of small pieces of seaweed, where overlooked water droplets can influence the results dramatically. While all seaweeds were treated with deliberate care (by dabbing fronds with a paper towel three times before and after weighing at a scale with four digits), there may have still been an effect of water weight. To get around this I would run an experiment with a much greater sample size. It would also be important to conduct similar experiments under more realistic field conditions (Thomsen et al. 2007). Despite these limitations, my study still demonstrated grazing on both the epiphyte and host, and indicated that the grazing communities probably have relatively small negative effects on the seaweeds that facilitate them (Figure 5.1).

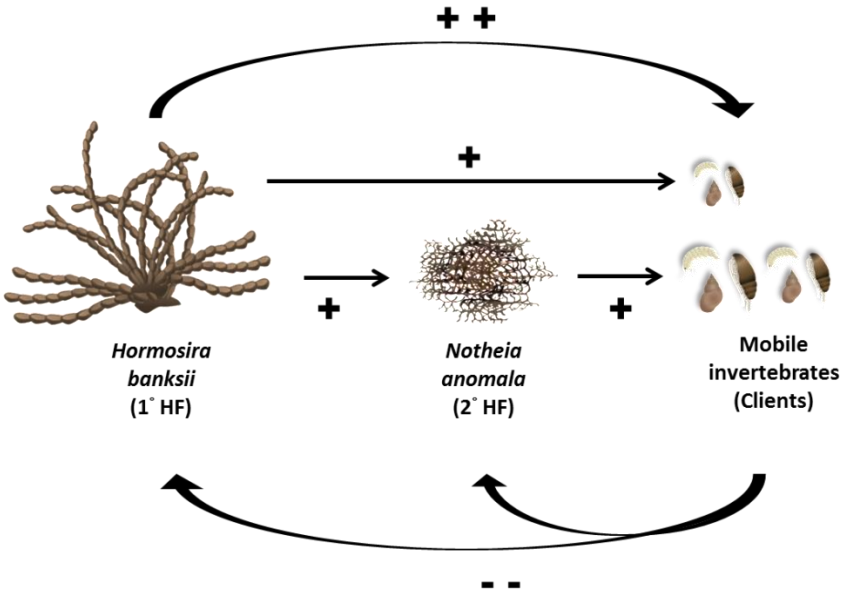

Figure 5.1. A diagram showing the positive effects of a habitat cascade, and the negative effects that invertebrates may be having on hosts that facilitate them.

In conclusion, the results of this study have consistently documented positive effects of the obligate epiphyte *Notheia anomala* on the diversity of small mobile invertebrates (inhabitants) on intertidal rocky reefs across the South Island of New Zealand. I found strong evidence that facilitation was strongest when *Notheia* biomass was greatest, and I demonstrated that the facilitative effect of *Notheia* is primarily due to the increased structure and complexity it provides to the system as opposed to being a food source. My results support a growing number of habitat cascade studies from different ecosystems, and suggest that these types of processes are common in marine benthic systems where epibiosis is a common occurrence.

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Appendix

Table A.1. Histology fixative recipes. Fixatives changed during the year; from October to April, the fixative was a sodium cacodylate-buffered glutaraldehyde solution, and from May to September a formalin/propionic acid solution.

Fixative	**Recipe**
Buffered Glutaraldehyde solution i.e. 5 % Glutaraldehyde in 0.1 M Sodium cacodylate buffer (made up in diluted seawater – final salinity 30 ppt), pH 6.8. Fixed at 4 ºC.	**(to make 200 mL of fixative)** 1) Sodium cacodylate buffer: a) 250 mL Seawater (1 µm filtered, diluted to salinity of 28 ppt). b) 0.1 M Sodium cacodylate (4.28 g in 200 mL above seawater) 2) Glutaraldehyde solution: 40 mL Glutaraldehyde solution (conc. 25%) 160 mL Sodium cacodylate buffer Change pH to 6.8
Formalin/Propionic Acid/Alcohol (FPA) Fixed at 4 ºC	**(to make 1L of fixative)** 615 mL Distilled water 305 mL Absolute Ethanol 30 mL Formaldehyde solution (conc. 37%) 50 mL Propionic acid

Dehydration:

Table A.2. Dehydration sequence performed manually, prior to processing in an automated tissue processor.

Reagent	Time HR:MIN	Temp.
Buffer/Distilled H_2O (2x rinses)	00:30	Room Temp.
Ethanol 50% (2x rinses)	00:30	Room Temp.
Ethanol 70%	01:00+	Room Temp.
Leave in 70% until further processing		4 °C

Table A.3. Dehydration sequence of samples placed in the automatic machine. (Samples have already been processed up to the 70% stage manually. See Table A.2).

Reagent	Time HR:MIN	Temp ºC	Pressure/Vacuum
Ethanol 70%	00:30	40	Ambient
Ethanol 95%	01:00	40	Ambient
Absolute Ethanol (1)	01:15	40	Ambient
Absolute Ethanol (2)	01:15	40	Ambient
Absolute Ethanol (3)	01:15	40	Ambient
50% Ethanol/Xylene	01:20	40	Ambient
Xylene (1)	00:45	40	Ambient
Xylene (2)	00:45	40	Ambient
Histosec wax (1)	01:20	60	Ambient
Histosec wax (2)	01:20	60	Vacuum
Histosec wax (3)	01:20	60	Vacuum

Staining:

Table A.4. Haematoxylin and Eosin solutions from Massey University (Evelyn Lupton, pers. comm.). Gills II Haematoxylin must be mixed in order, and all solids must be dissolved before adding acid. Can be used at once, but acid content needs to be maintained with one drop per 100 mL weekly (Make fresh solution monthly). The Eosin/Phloxine working solution must be fresh fortnightly.

Stain	**Recipe**
Gill's II Haematoxylin (Fresh monthly)	4 g Haematoxylin 700 mL Distilled water 250 mL Ethylene glycol 0.6 g Sodium iodate (anhydrous) 70.2 g Aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) 50 mL Acetic acid (glacial)
Eosin/Phloxine stock solution	1 g Eosin Y (C.I. 45380) 0.1 g Phloxine B 110 mL Distilled water
Alcoholic Eosin/Phloxine working solution (Fresh fortnightly)	110 mL Stock Eosin/Phloxine 880 mL 95% Ethanol 5 mL Acetic acid (glacial)
Scott's Tap Water	50 g Magnesium sulphate ($MgSO_4.7H_2O$) 2 g Potassium bicarbonate ($KHCO_3$) 2 L Tap water

Table A.5. Protocol for the manual Haematoxylin and Eosin/Phloxine staining sequence. (Adapted from Massey University, Evelyn Lupton, pers. comm.). The washes were done under running tap water.

Step	Station	Reagent/Process	Time (min:sec)	Exact
1		Oven (37°C)	10:00	Y
2	1	Xylene	3:00	Y
3	2	Xylene	2:00	N
4	3	Absolute Ethanol	1:00	N
5	4	Absolute Ethanol	0:30	N
6	5	70% Ethanol	1:30	N
7		Wash 1	1:00	N
8	7	Gill's (II) Haematoxylin	4:00	Y
9		Wash 2	0:30	N
10	6	Scott’s Tap water	0:30	Y
11		Wash 3	1:00	N
12	8	Eosin/Phloxine	2:00	Y
13		Wash 4	0:30	N
14	13	70% Ethanol	0:10	Y
15	14	95% Ethanol	0:15	Y
16	15	Absolute Ethanol	0:45	N
17	16	Absolute Ethanol	1:00	N
18	17	Xylene	1:00	N
19	18	Xylene	1:00	N
20		End - Coverslip		